Laboratory Manual for
INTRODUCTORY MYCOLOGY

by

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Introduction

This manual is designed for introductory mycology courses which undertake a general survey of the fungi. The writers believe that a general knowledge of the morphology and taxonomy of the fungi is fundamental before students are plunged into the more glamorous phases of experimental mycology and have, therefore, chosen to organize this manual along traditional lines.

The morphology, life history, and taxonomy of the fungi can be made exciting to students if good, living material is made available at the proper stage of development. There are few students who will not become excited when myxomycete spores germinate under their lenses and the swarm cells begin their gyrating movements; when Allomyces releases its gametes before their eyes and copulation results in the formation of zygotes; or when the vesicles of Pythium develop and the zoöspores break out of the all but invisible vesicular membrane. The secret lies in providing excellent and abundant laboratory material at all times. This requires considerable preparation on the part of the instructor, but the results are most gratifying.

The manual has been revised to conform with the second edition of Alexopoulos' INTRODUCTORY MYCOLOGY which it is designed to accompany. Part I has been expanded to incorporate new techniques for the study of fungi and to include a list of available Educational Films which may be used to illustrate phases of the structure or development of various fungi which are difficult to illustrate by other methods.

In Part II, which constitutes the main body of the manual, the Outline of the Achlorophyllous Thallophytes, has been maintained, revised, and expanded to conform with the newer classification. Students have found this to be a useful review checklist and guide to the interrelationships of the fungi they have studied during the course.

The form in which the first edition was written has been maintained. Some material has been added, however, and the selected references at the end of each section have been greatly expanded to incorporate the more recent research articles.

The authors hope that both teachers and students will continue to find the manual useful and will welcome suggestions for the improvement of subsequent editions.

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Everett S. Beneke

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Part I

Useful Methods for the Study of Fungi

In the study of fungi it is usually necessary to make either temporary or permanent mounts of various portions of the material to be used for microscopical examination. The following are simple methods that are frequently used:

I. Preparation of Materials for Mounting

Many fungi may be mounted without sectioning. Portions of the somatic and reproductive structures may be removed from culture or from the natural substratum and placed in a drop of the mounting medium on a glass slide. Freehand sections of larger fungous fruiting bodies should be made for the observation of structural details. In case the fruiting body is small, or the fungus is growing on a leaf, it is frequently necessary to place a piece of split pith around the material before making sections. The material can then be sliced with a sharp razor blade and the sections may be placed on a drop of the mounting medium.

II. Slide Preparations

A. MOUNTING FRESH MATERIAL. Small fungus structures, including sections, may be placed directly into a drop of distilled water or mounting media, such as lactophenol, on a slide if the material is fresh. A weak detergent or soap solution used instead of water will help to reduce bubbles. The cover-slip should be lowered slowly and at an angle onto the drop of water. If this is done carefully the slide will be free of air bubbles.

When lactophenol or a glycerin-containing mounting medium is used, the slide and cover glass may be sealed with nail polish or other sealing materials. When the mounting medium is placed on the slide excessive amounts should be avoided so that the liquid will not extend beyond the edge of the cover glass. If liquid is present around the edges of the cover glass, then the excess should be wiped off before sealing the edges.

B. MOUNTING DRIED MATERIAL. When a small portion of a dried fungus is to be mounted, a weak solution of KOH (2-10%) is used in place of a drop of water on the slide, in order to wet and swell these structures to their natural size. Dried, sectioned material should be mounted in KOH in a similar manner. Gently heating the slide will increase the rate of swelling and clearing of the material.

Another method which is very satisfactory is mounting the dry material in a drop of lactophenol on a slide, covering with a cover-glass and heating over a lighted match or small bunsen burner flame until the mounting medium begins to simmer. The slide should be thoroughly cooled before placing on the microscope stage for observation.

If the material is opaque, as is the case with dried leaves, small portions may be boiled for a few seconds in 10% KOH in a test tube, to remove a part of the brown pigment, and mounted in water on a slide.

C. MOUNTING MEDIA

1. Amman's mounting medium (lactophenol). The following mounting medium is very satisfactory for both temporary and more permanent type slide mounts of most fungi. Amann's medium contains the following ingredients:

Phenol	20 gm.
Lactic acid	20 gm.
Glycerine	40 gm.
Distilled water	20 gm.

If it is desirable to have the protoplasm stained, a 0.05% to 0.1% solution of cotton blue, brilliant green, eosin, phloxine or other suitable dye may be added to the lactophenol.

2. Glycerine jelly. This medium has been used as a very satisfactory solution for mounts of fungi. This medium is made as follows:

Gelatin	1.0 gm.
Glycerine	7.0 gm.
Water	6.0 gm.

Add .14 gm. phenol to the above solution.

3. Hoyer's mounting medium. This medium has been used frequently for the mounting of insects. It also has been used very successfully for mounting Myxomycetes and a number of Ascomycetes by Dr. R. K. Benjamin. The medium contains:

Distilled water	50 gm.
Arabic gum lump	30 gm.
Chloral hydrate	200 gm.
Glycerine	20 gm.

Arabic gum should be soaked in water for about 24 hours. Add the chloral hydrate and let the solution stand until all the material dissolves. This may require several days before the glycerine is added and the solution is ready for use. For mounting Myxomycetes or Ascomycetes wet the specimens with absolute alcohol for about one minute, treat with 2% KOH for a similar period of time, wash in 70% alcohol, and place in a drop of mounting fluid. Cover with a cover-slip.

Other procedures may be used in the preparation of permanent slides. Some of these will be found in the references on methods.

D. HANGING DROP SLIDES: Slides with a concave depression in the center may be used for studying germination of spores in hanging drops. A drop of liquid is put on a cover-slip, inverted and placed over the depression in the slide. This will form a hanging drop which may be observed through the cover-slip. To reduce evaporation vaseline should be rubbed around the edges of the depression before placing the cover-slip on the slide. Van Tieghem rings and flat slides may be used when concave slides are not available, or when a larger hanging drop is desired. Van Tieghem rings are glass rings of various diameters and heights, which are secured on a flat slide by means of vaseline or other materials. A little distilled water is placed in the ring which supports the hanging drop prepared as described above.

E. SLIDE PREPARATIONS TO SHOW FLAGELLA: In many of the lower forms of fungi it is desirable to determine what types of flagella are present. The following technics may be used for observation of flagella:

1. Osmic acid fixation. After a drop of water containing zoöspores is placed on a slide, the slide is inverted over a drop of 1% osmic acid for about one-half minute. To stain, place a small particle of gentian violet on the droplet and add the cover-glass.

2. Lugol's iodine. Place a drop of Lugol's iodine on a suspension of swarm cells and observe the flagella.

3. Bouin's solution. The flagella of *Achlya sp.* or *Saprolegnia sp.* may be observed by placing a drop of Bouin's solution with a drop of water containing zoöspores, and adding a cover-glass to the slide. [Bouin's solution: Saturated aqueous picric acid, 300 ml.; formalin (40% commercial formalin), 100 ml.; and glacial acetic acid, 20 ml.]

4. Phase contrast microscopy. Place a water mount containing zoöspores of *Achlya*, *Saprolegnia*, or other genera that produce zoöspores, under the phase contrast microscope and observe the action of the zoöspores, and the flagella.

F. CALIBRATION OF THE MICROSCOPE

The microscopist frequently wishes to measure the size of the objects in the microscopic fields. The apparatus used in such work is an ocular micrometer, a disc of glass with an engraved scale usually containing 50 accurately spaced divisions. Since there is no standard value for each division, the divisions must be calibrated for each ocular and objective, using a stage micrometer for the determinations.

Procedure:

1. Place the ocular micrometer with ruled side of disc up in the ocular. Place the stage micrometer on the stage of the microscope.

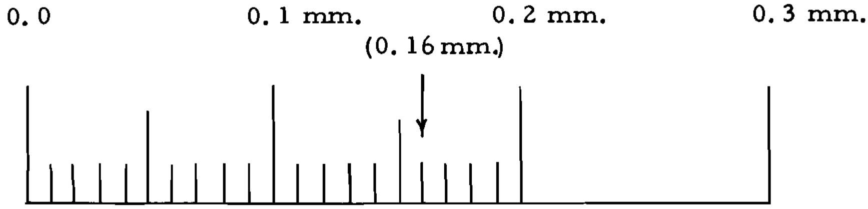
2. Focus and arrange the ocular micrometer so that the ruled scale coincides with the scale of the stage micrometer. Move the stage micrometer slide until the smaller divisions (0.01 mm.) are next to the ocular micrometer.

3. Adjust the first line of the ocular micrometer so that it coincides with a large division line and the other end of the ocular eyepiece scale or one near the end will be superimposed on the smaller division of the stage micrometer.

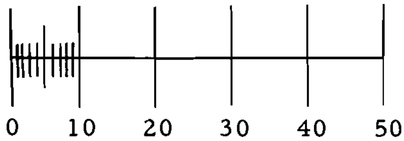
4. If, as an example, the 10x ocular and the 44x objective are used, then the 50 divisions of the ocular micrometer could extend through one large space and six small spaces of the stage micrometer. Thus 50 divisions of the ocular micrometer would equal 0.16 mm. on the stage micrometer.

5. Since 1 mm. equals 1000 microns (μ), the 0.16 mm. equals 160 microns. Therefore, each division on the ocular micrometer scale will equal 3.2 microns when 160 microns is divided by 50 divisions of the ocular micrometer scale.

Stage Micrometer Scale:



Ocular Micrometer Scale (50 Divisions):



$$\frac{0.16\text{ mm.}}{50\text{ spaces}} = \frac{160\mu}{50} = 3.2\mu$$

The calibration of lower or higher power objectives and eyepieces should be done in a similar manner, since, the divisions of the ocular micrometer will have different values at different magnifications.

III. Special Techniques for the Study of Slime Molds

A. COLLECTION. Since the fruiting bodies of slime molds are small and delicate it is desirable to have small boxes or compartments in a basket for the collection of fresh specimens. A simple and effective collecting kit may be improvised by purchasing a rectangular, metal, picnic basket with cover, and stacking a sufficient number of plastic silverware containers, with four compartments each into the metal basket. A scratch pad, a pencil, a hand lens, and a sharp jack-knife, as well as a small bottle of mosquito repellent can also be placed in the box along the stack of plastic trays.

Each specimen should be placed on a piece of paper bearing a number, the date and place of collection, and any field notes which are desirable to make at the time of collection. After the specimens are brought into the laboratory, they should be air dried before mounting in individual boxes. For final mounting, rectangular pill boxes may be purchased or match boxes may be used. The specimens are secured in the boxes with glue, and the data typed or printed on labels which are glued to the outside of the boxes for ready reference. If possible, labels and specimens should be on the same part of the box.

B. CULTIVATION. Slime molds, particularly certain species of Didymium, Fuligo, Physarum, and Stemonitis, may be cultivated on agar media, filter paper, or sterilized bark of trees, and be induced to complete their entire life cycle in culture. Freshly collected specimens are best for such work.

Prepare half-strength corn meal agar (equal quantities of Difco corn meal agar without glucose and 2% plain agar) and pour 15-20 mls. in each Petri dish. With a pair of sharp forceps (jeweler's forceps with finely ground tips) grasp fruiting body by the stalk and rub the sporangial head on the agar to spread the spores. Add 2-4 mls. of sterile distilled water and one drop of a heavy suspension of actively growing Escherichia coli or Aerobacter aerogenes. Addition of the bacteria is not necessary but will accelerate development. In four to six days plasmodia will have been formed on the agar if the species selected is easily cultivable. When tiny plasmodia appear, sprinkle a small quantity of sterile (autoclaved) pulverized rolled oats on the culture.

Filter paper, towelling paper, or sterilized bark of trees may be used as a substratum instead of agar. Addition of bacteria to this type of culture is essential, and observations are much more difficult because such substrata are opaque.

Slime molds may be maintained growing in culture by periodic transfers of a portion of the plasmodium to fresh medium. This is especially true for Physarum polycephalum which is one of the easiest slime molds to grow. Oatmeal agar is one type of medium that may be easily prepared. It contains 30 grams of rolled oats, 15 grams of agar, and 1000 milliliters of distilled water.

Another successful method for cultivating plasmodia is the following: Two large finger bowls, one on top of the other will serve as a moist chamber in which to grow the plasmodium. Put a little water into the bowl and place an inverted Petri dish covered over with filter paper on the bottom of the finger bowl so that the filter paper acts as a wick. Place a portion of the plasmodium on the filter paper, which has become moist from absorption of water, and sprinkle some rolled oats on the surface of the filter paper. The plasmodium will feed upon the oat meal and increase in size. More rolled oats should be added every few days. Transfers to clean filter paper should be made at intervals of a week or ten days.

For fruiting, the plasmodia should be transferred to a weak medium (half-strength corn meal agar, Knop's agar, or plain agar) or to clean filter paper, and allowed to spread without being fed. They should be exposed to daylight or artificial light at least a part of the day.

C. A SIMPLE METHOD FOR OBSERVING PROTOPLASMIC FLOW IN A PLASMODIUM. Prepare a weak agar medium containing 3 grams of maltose, 1 gram of peptone, and 20 grams of agar per liter of water. After autoclaving for 15 minutes at 15 lbs. pour into sterile Petri dishes so that a very thin film of agar covers the bottom of each plate and appears nearly transparent. Place a small piece of the plasmodium on the surface of the agar medium 12 to 24 hours before demonstration time.

During this period the plasmodium will spread over the agar and the flowing of the protoplasm in the plasmodial veins may be easily observed by inverting the Petri dish on the microscope stage and observing through the bottom by means of the low power objective.

IV. Special Techniques for the Study of Fungi

A. CULTURE OF FUNGI

1. Cleaning glassware. For routine cleaning of glassware washing with a detergent solution is usually sufficient. In sections of the country where the mineral content of water is high, distilled water should be used for rinsing the glassware. When the glassware bears wax pencil marks, paraffin, balsam or similar materials, xylol may be used effectively for removing these before the glassware is washed.

   If the glassware is very dirty, it is necessary to use cleaning solution. The use of cleaning solution is also recommended when it is necessary to have the glassware exceptionally clean. Cleaning solution is prepared as follows:

Potassium bichromate	100 gm.
Sulfuric acid	500 mls.
Water	1000 mls.

Thoroughly dissolve the potassium bichromate in hot water. Cool the solution and slowly add the sulfuric acid stirring constantly. Great care should be exercised in handling the cleaning solution as it will break down cloth and will injure the skin. Soak the glassware in cleaning solution for several hours, clean thoroughly with a brush, and rinse several times, first in tap water and finally in distilled water.

2. Types of media for growing fungi. Fungi may be cultured on a variety of substrata or media. Such media may be either liquid or may be made solid by the addition of agar. Most fungi will grow well in media high in carbohydrates, with a pH range between 5 and 6, whereas bacteria usually grow better in media containing more proteins, and with a pH in the proximity of 7. There is no one medium ideally suited for the culture of all fungi, for the nutritional requirements vary considerably with the species. Some fungi will grow on almost any medium which contains some organic matter and a sufficiency of moisture; others require media of special chemical composition. Some fungi, such as downy mildews and powdery mildews, have not as yet been grown successfully on artificial media. Classified on the basis of their composition, there are two main groups of culture media: natural media, which contain infusions of natural substances the chemical composition of which varies from time to time, and synthetic media which contain ingredients of known chemical composition. Natural media cannot be duplicated exactly; synthetic media, on the contrary, can be duplicated with a high degree of accuracy. A few of the most common media which are frequently employed in routine culture work are described below. These may be prepared in the laboratory or may be purchased in dry powder form ready to mix with water and sterilize.

a. Natural media

(1) Corn meal agar. Satisfactory for the growth of many fungi; often induces sporulation.

Corn meal	20 gm.
Peptone	20 gm.
Dextrose	20 gm.
Agar	15 gm.
Distilled water	1000 mls.

The peptone and the dextrose may be omitted from the above formula. Add the corn meal to the water and simmer in a water bath for one hour. Filter through coarse filter paper using a Buchner funnel, or decant. Add the agar and, if desired, the other ingredients, and melt in the autoclave or the Arnold sterilizer. Filter through two layers of cotton held in gauze. Tube and sterilize for 15 minutes at 15 lbs. in the autoclave.

(2) Oat flake agar. One of the most satisfactory and inexpensive media to prepare for routine maintenance of cultures is oat flake agar. Prepare $1\frac{1}{2}\%$ water agar (see formula 8 below) in a flask. Fill with oat flakes the bottom half-inch of each test tube to be used, and pour 10-15 ml. water agar over the flakes. Plug, autoclave, and slant (see section 3 on the following page).

(3) Sterilized corn stalks. Many fungi which do not fruit on agar media do so readily on sterilized corn stalks. Cut thin strips of corn stalks about four inches long and place them one in a culture tube. Add 1-2 ml. distilled water, or preferably of a salt solution (see formula b, 1 below, but omit the sucrose and the agar) to each tube, plug, and autoclave.

(4) Prune agar. Useful for many fungi; not satisfactory for bacterial growth.

Dried prunes	40 gm.
Agar	20 gm.
Distilled water	1000 ml.

Preparation as for corn meal agar.

(5) Potato dextrose agar. Satisfactory for the growth of many fungi, especially phytopathogens, and some bacteria.

Peeled, diced potatoes	200 gm.
Dextrose	20 gm.
Agar	15 gm.
Distilled water	1000 ml.

Sucrose (10 gm.) may be substituted for dextrose. Preparation as for corn meal agar.

(6) Malt extract agar. Very useful for the growth of wood-destroying and many other fungi.

Malt extract	25 gm.
Agar	15 gm.
Distilled water	1000 ml.

Dissolve the ingredients in the water by placing the flask containing them in the Arnold sterilizer. Tube and autoclave for 15 minutes at 15 lbs.

(7) Alphacel medium. This medium (Sloan, et al., 1960-1961) and the modified formula below are especially good for reduced mycelial growth and greater sporulation of the fungi. This is useful for the identification and nutritional studies of the fungi.

Alphacel*	20 gm.
$MgSO_4$	1 gm.
KH_2PO_4	1.5 gm.
$NaNO_3$	50 gm.
Coconut milk	50 ml.
Distilled H_2O	1000 ml.

Adjust the pH to 5.6; sterilize 20 minutes at 20 lbs.

*Modified alphacel medium contains the addition of tomato paste, 10 gm., and 10 gm. of Beech-Nut baby oatmeal.

(8) Water agar. This medium supports only sparce growth of fungi. It is useful as a base for placing about a cubic centimeter of animal dung on the surface for observation of various fungus successions that develop.

Agar	20 gm.
Distilled water	1000 ml.

(9) Waksman's medium. This is a special medium for counting soil fungi.

Glucose	10 gm.
Peptone	5 gm.
KH_2PO_4	1 gm.
$MgSO_4 . 7H_2O$	0. 5 gm.
Agar	20 gm.
Distilled water	1000 ml.

Adjust the pH to 4 by adding normal H_2SO_4 or H_3PO_4

(10) Yeast starch Agar (YpSs). This medium is particularly suitable for maintaining Allomyces as well as other fungi.

Yeast extract	4 gm.
Soluble starch	15 gm.
K_2HPO_4	1 gm.
$MgSO_4 . 7H_2O$	0. 5 gm.
Agar	20 gm.
Distilled water	1000 ml.

(11) Sabouraud's agar. This medium will support the growth of many fungi including human or animal pathogens.

Glucose (or Maltose)	40 gm.
Peptone	10 gm.
Agar	15 gm.
Distilled water	1000 ml.

(12) Krainsky's medium. This is useful for cultivation of actinomycetes.

Glucose	10 gm.
Asparagin	0. 5 gm.
K_2HPO_4	0. 5 gm.
Agar	15 gm.
Distilled water	1000 ml.

(13) Bread crumb agar. This medium is useful for demonstration of luminescence in some of the dikaryotic cultures of Panus stipticus, Armillariella mellea, and Mycena polygramma or other Basidiomycetes that show luminescence. For additional information see Berliner (1961).

Bread crumbs	100 gm.
Agar	18 gm.
Distilled water	1000 ml.

After fourteen days, up to 21 days, the dikaryotic mycelial cultures that are maintained at 22° C. in the dark exhibit the highest light intensity.

(14) Yeast-Malt extract medium. This medium is useful in demonstrating sexual agglutination in certain yeasts (L. J. Wickerham).

Yeast extract	3 gm.
Malt extract	3 gm.
Peptone	5 gm.
Glucose	10 gm.
Agar	20 gm.
Distilled water	1000 ml.

The pH is not adjusted.

For shake cultures to demonstrate sexual agglutination 30 gm. of glucose is used instead of 10 gm. and no agar.

(15) Beef extract agar. Useful for the isolation of bacterial plant pathogens, and for the growth of many bacteria.

Beef extract	3 gm.
Peptone	5 gm.
Agar	15 gm.
Distilled water	1000 ml.

Adjust the medium to a final pH of 6. 2 to 7, remembering that the pH will be lowered by autoclaving.

(16) Hemp seeds. Certain types of aquatic fungi, namely, members of the Saprolegniales and the Leptomitales, are commonly cultured on halves of boiled hemp seeds placed in water from ponds or streams. If hemp seed is not available, corn, wheat, or dead insects may be utilized.

(17) Miscellaneous media for aquatic fungi. Many of the Chytridiales inhabiting pond waters have been grown successfully on pieces of cellophane, filter paper, hair, feathers, bleached young corn leaves, onion skin, pollen, or other organic substrata.

b. Synthetic media

(1) Czapek's solution agar. Especially suitable for growing *Aspergillus, Penicillium* and *Nocardia* sps.

$NaNO_3$	3. 00 gm.
K_2HPO_4	1. 00 gm.
KCl	0. 50 gm.
$MgSO_4 . 7H_2O$	0. 50 gm.
$FeSO_4 . 7H_2O$	0. 01 gm.
Sucrose	30. 00 gm.
Agar	15. 00 gm.
Distilled water	1000. 00 ml.

The sugar should be added just before final sterilization in order to reduce caramelization.

For more detailed information on special kinds of media, adjustment of pH, and other special procedures, consult one or more of the references listed at the end of this section.

Where time is a factor, or only a small quantity of media is required, it is very convenient to have various kinds of media in tablet form. A tablet dissolved in 10 ml. of water makes up one tube of medium. The cost of such tablets is somewhat higher than the cost of the ingredients which they contain, but their use does save much labor. Various media in tablet form may be purchased from the Ben Venue Laboratories, Inc. (see page 18).

3. Preparation of media and sterilization

a. Preparation of media. The preparation of media will vary somewhat according to the kind used, but the general routine procedure is similar for most media. When media which are purchased as dry powders are used the procedure for their preparation is outlined on the label of the container.

Preparation of media usually involves weighing the designated quantities of ingredients, dissolving them in the required amount of distilled water in a flask, distributing the media into test tubes, usually in 10 or 12 ml. quantities, plugging the tubes with cotton, or capping them with plastic or metal caps, and sterilizing. All glassware should be thoroughly cleaned and rinsed in distilled water. Cotton plugs should be tight. If large quantities of media are prepared for use over a relatively long period of time the tubes may be placed (1) in quart Mason jars with a little water at the bottom of each jar, sealed, and autoclaved, or (2) in plastic bags stored in a refrigerator. The media will then last indefinitely without drying.

Plastic bags may also be used for storing Petri dishes containing agar media.

b. Sterilization. Since media are prepared in order to grow fungi in pure culture, all microorganisms, other than the one to be grown, should be excluded. This makes it necessary to sterilize the media before using in order to kill any bacteria or fungal spores which are present in the media or in the glassware containing them. Sterilization is accomplished by placing the media in an autoclave or a pressure cooker and steaming them for 15 or 20 minutes at 250° F. and 15 lbs. pressure. If a very large volume of liquid is being sterilized the time must be increased correspondingly. Media which contain sugars which will hydrolize at autoclave temperatures should be sterilized by steaming at 100° C. (live steam), in an Arnold sterilizer, for one hour each day on three successive days.

Glassware, such as Petri dishes, may also be sterilized in the autoclave to save time, but since they come out wet and spotted, it is much better to sterilize them by dry heat in a hot-air oven. Glassware may either be wrapped in wrapping paper, newspaper, or towelling or, better still, may be put in metal containers before placing in the oven. A temperature of 150° to 160° C. for 3 or 4 hours is generally sufficient for dry sterilization of glassware.

4. Pouring plates. After the Petri dishes and the media have been sterilized we are ready to pour the contents of one or two tubes of media, depending on the quantity desired, into each Petri plate. First, wet the surface of the table with a detergent, a disinfectant, or even with plain water, and close the windows and doors to reduce air currents to a minimum. Place the Petri dishes on the table, light a bunsen burner and proceed as follows:

a. Take a tube of melted medium
b. Remove the cotton plug with your left hand
c. Flame the mouth of the tube
d. With your left hand raise one side of the Petri dish cover just enough to insert the mouth of the tube, and pour all the medium into the plate
e. Replace the cover immediately and gently rotate the dish so as to spread the agar evenly over the bottom of the dish
f. Allow the plates to cool sufficiently for the medium to harden, before using.

5. Agar slants. An agar slant is prepared by permitting the agar in a tube to solidify while the tube is in a slanted position. During slanting care should be taken not to permit the melted agar to come in contact with the cotton plug.

B. ISOLATION AND GROWTH OF FUNGI

1. Isolation. Fungi should be studied, as far as possible, in their natural habitats, but some fungi are so difficult to observe when intermingled with others that a pure culture becomes necessary. Pure cultures are also necessary in order to determine whether two fruiting stages suspected of belonging to the same fungus are actually produced by the same colony resulting from a single spore. Finally, pure cultures are employed in plant or animal pathology to prove the causality of a disease through Koch's postulates, and are absolutely necessary for physiological and genetic experiments.

   a. Isolation of mushrooms. Many mushrooms may be cultured by taking the cap of a fresh mushroom, after cutting off the stalk, and splitting it through the center. With sterile forceps remove a small amount of the tissue from the fleshy part of the cap and culture on malt agar or acid potato dextrose agar.

   b. Plant disease. The surface of a plant that is diseased should be disinfected with about 1 to 4 dilution of ordinary laundry bleach (containing sodium hypochlorite). Cut the diseased part of the plant into small pieces and place in the disinfectant for about one minute. Wash in sterile water, and plant several pieces on malt or acid potato dextrose agar for observation.

   c. Isolation of saprobes. The common saprobic fungi, such as *Penicillium*, *Aspergillus*, and *Saprolegnia*, grow readily in standard culture media. Many coprophilous forms grow on sterilized dung or dung agar, and aquatic forms grow on decaying twigs, dead insects, or other organic matter. As mentioned before, some fungi--considered at present to be obligate parasites--cannot be grown in culture.

   Fungal colonies may be started very easily on agar plates by removing the cover of a Petri dish for a couple of minutes and exposing the sterile surface of the agar to the air in which fungal spores are usually floating.

   Spores which land on the surface of the agar grow and give rise to colonies from which pure cultures can be easily obtained by making transfers onto a sterile agar slant or onto the agar in a Petri dish, using the method described in a subsequent paragraph.

Stray spores frequently invade Petri dishes or slants in which pure cultures are growing and give rise to colonies, thus "contaminating" the pure cultures. Such stray colonies are known as contaminants.

d. Isolation of aquatics. Certain members of the Saprolegniales and Leptomitales are usually cultured on halves of boiled hemp seeds placed in rain water or distilled water. As mentioned in a previous paragraph, the original isolation of such fungi is accomplished rather easily by placing half a hemp seed in water collected in a pond or a stream. Colonies of aquatic fungi will develop around such seeds and can then be purified from the contaminants which often grow associated with them.

To clean a hemp seed culture of an aquatic fungus from the associated bacteria, cut off tips of hyphae and place them on a weak agar medium containing:

Maltose	3 gm.
Peptone	1 gm.
Agar	20 gm.
Distilled water	1000 ml.

Instead of using hyphal tips, single spore isolations may be made by streaking a drop of water containing the zoöspores of the fungus across the surface of the agar in the Petri dish. After the fungus has grown out sufficiently on the agar so that the hyphal tips are clear of bacterial contamination, small blocks of the agar, about one centimeter square, containing the fungal hyphae, may be cut out and placed in a sterile Petri dish. Sufficient sterile distilled water (preferably treated with charcoal to remove impurities) is added to bring the water line up to the upper edge of the agar block. Half of a boiled hemp seed is now placed on top of the agar block. In a day or two, more distilled water may be added to submerge the hemp seeds. Actively growing colonies will develop in a few days illustrating the formation of zoöspores and of sex organs.

e. Additional materials for isolations. The addition of such materials as cellophane, filter paper, rosaceous fruits, and keratinous material to the water from ponds and streams will induce the growth of fungi belonging to the Chytridiales and Blastocladiales.

Special techniques which have been developed for the isolation of pathogenic fungi are described in some detail in the references listed at the end of this section.

2. Transferring fungi to slants and Petri dishes. Fungal cultures are perpetuated in the laboratory either by transferring spores from one culture to sterile media, or by transferring fragments of the mycelium which develop into new colonies. All transferring should be done under sterile conditions as follows: Use an inoculating needle consisting of a long handle supporting a given length of chrome or platinum wire. The tip of the wire may be bent into a hook or made into a closed loop. Heat the wire in the flame of a bunsen burner until it becomes nearly white-hot; also pass as much of the handle as possible through the flame quickly in order to kill any microorganisms that may be present on its surface. Remove the plug from a slant culture of the organism to be transferred and also from a sterile agar slant.

Both plugs must be held between the fingers while the open ends of the test tubes are flamed to kill any stray spores or bacteria. Never permit the cotton plugs to come in contact with any object while transferring the fungus. After the wire is cool, remove some spores or a small portion of the mycelium from the slant culture and gently transfer it to the sterile slant. Work fast but gently; violent movements create air currents which may introduce contaminants into the open test tubes. Now flame the ends of the two tubes again before replacing the cotton plugs. Heat your needle again to kill any spores that remain on it, before you put it away. After a few days the spores or the mycelial fragment which was transferred to the new slant will have developed into a colony. If care was taken in making the transfer and the air in the room was quiet, the new slant should be free of contamination.

When fungi are to be transferred into Petri plates, the technique is similar to that described above. In order to reduce contamination, the Petri dish cover should be opened no more than necessary while inoculating the agar medium. The inoculated plates should be stored upside down while the colonies are developing.

3. Demonstrating fungal growth in the laboratory. The large, dark ascospores of Anthostomella, Sordaria, Gelasinospora, or indeed, of any rapidly growing fungus are ideal for demonstrating growth during the usual two-hour laboratory period. Place several ascospores on an agar medium in a Petri dish about 8 to 12 hours before observation is to begin. In order to observe growth, uncover the Petri dish and place a thin cover glass on a germinating ascospore. Observe at intervals of 15 minutes over a two hour period and note growth of the germ tube, formation of branches, septa, and other structures. Since the percentage of ascospore germination in the genera mentioned and in many others is low, heating the ascospores at the time of sowing is advisable. To do this place the Petri dishes containing the ascospores into an electric oven and heat to 70° C. gradually, taking about 30 minutes to reach that temperature. As soon as the thermometer approaches 70° C. turn off the current and allow the Petri dish to remain in the oven until the temperature drops to 30° C. or below. Remove from the oven and incubate at room temperature until demonstration time.

4. Hair baiting technic for keratinophilic fungi (Vanbreuseghem (1952): Samples of soil and other materials may be collected in four ounce bottles or plastic bags for laboratory study. Types of habitats where keratinophilic fungi have been isolated are: animal burrows; animal carcasses; basement soil; interior and adjacent chicken house soil; dog pen soil; feed lot soil; garage soil; garden and yard soil; animal and human manure; tobacco barn and bed soil; and decaying wood and vegetation.

   a. Fill sterile Petri dishes half full with soil sample. Add 15 to 30 ml. of sterile distilled water to the soil so that some excess water is present. Small clear areas may be made in the soil.

   b. Scatter short strands of sterilized human or horse hair over the surface of the soil. If small clear areas are made, and the hair extends over the clearings in the Petri dish, observations may be made in a few weeks directly under the low power objective of the microscope for the development of hyphae along the hair shaft.

   c. Incubate the preparations at room temperature (20-25° C.) in the dark for periods up to 4 or 6 weeks.

d. Examine the hairs periodically for the development of mycelium on the sides of the filament.

e. Remove hairs with fungus growth and place in tubes or plates of Sabouraud agar (containing cycloheximide and chloreamphenicol; if pathogenic fungi are to be studied).

f. After one or more weeks check the colonies and identify the genus and species. Pure cultures may be made by transferring single conidia to new media.

5. Slide cultures for studying fungi. The growth of fungi in a van Tieghem cell or in culture chambers has been used for a more detailed study of the fungi. An improved method for obtaining permanently stained slides from a slide culture has been published by Riddell (1950). This very useful method is as follows:

   a. Pour about 10 ml. of melted agar medium into a sterile Petri dish.
   b. After solidification, mark the medium rapidly into 1 cm. squares using a sterile dissecting needle and a flamed glass rod.
   c. By means of sterile forceps place a flamed microscope slide under a Petri dish cover.
   d. Lift out an agar square and place it on the flamed slide.
   e. Inoculate the four sides of the agar block with spores or mycelial fragments of the fungus to be grown.
   f. Place a flamed cover-slip centrally upon the agar block.
   g. Transfer the slide culture into a moist chamber containing a small amount of 20% glycerine to prevent fogging of the slide. Glass rods or a piece of hardware cloth with the ends bent down should be placed in the Petri dish and the slides supported thereon to keep the slide culture from getting wet.
   g. Check the culture periodically for growth and sporulation.

   After sporulation has occurred, two permanent stained slides may be obtained from the slide culture by proceeding as follows:

   h. Remove the cover-slip from the agar block and apply a drop of 95% alcohol to the center of the cover-slip in order to wet the fungus.
   i. When the alcohol is nearly dry add a drop of lacto-phenol containing cotton blue, and lower the cover-slip gently upon a clean slide.
   j. Similarly, using the slide with the fungus growing on it, proceed as in steps (h) and (i) and cover with a clean cover-slip.

   If the correct amount of mounting medium has been used, the slides may be sealed immediately with finger nail polish as described in a previous section. Otherwise, it is necessary to let the slides dry overnight and then to absorb the excess medium by blotting paper before sealing.

6. Modified cellophane culture technic. This method of culture of colonies is useful for photographing and preserving of fungi for future reference. This technic published by Kondo et al in 1959 is as follows:

   a. Alternate 7 cm. cellophane discs between filter papers and place in a deep Petri dish.
   b. Autoclave dry at 15 lbs. for 20 minutes
   c. Dip individual sterile cellophane discs into sterile distilled water with sterile forceps, and arrange them on the surface of a 2.5% plain medium in a Petri dish.

d. If bubbles are present, raise the edge of the cellophane disc and force them out by rolling the disc back on the agar surface.
e. Allow the prepared plates to incubate 48 hours before inoculating.
f. Inoculate small biscuits of the inoculum onto the cellophane disc, then remove after growth starts, or inoculate a small amount of the fungus on the cellophane disc keeping the Petri dish inverted while the colony develops.

The colonies may be preserved as herbarium specimens by the cellophane discs on a labeled card and storing in a container. Details of this procedure are given in Kondo et al. (1959).

V. Collection and Preservation of Fungi

A. COLLECTION OF FUNGI. Collecting methods and equipment vary with the type of fungi to be collected. For small fruiting bodies growing on bark, on wood, on soil, or on other material which is not easily pressed, a vasculum, a market basket or a picnic basket equipped with plastic trays, as described for collecting slime molds, are very useful. Larger specimens, such as mushrooms, are generally wrapped individually in wax paper or tissue paper and placed in a market basket or in a picnic basket with a cover. Leaves or herbaceous stems on which parasitic fungi are growing may be placed in a vasculum or, better still, may be pressed between newspapers in a plant press as soon as they are collected. The latter procedure has the advantage of keeping individual collections separate from the start affording little opportunity for the spores on different collections to mix.

On the other hand, it has the disadvantage of necessitating the carrying of a bulky plant press in the field instead of the more easily handled vasculum, and of delaying collecting procedures.

Each collection should be accompanied by a slip of paper bearing a number, the place and date of collection, the host--in the case of parasitic fungi--and any other field notes of interest.

The following is a more or less complete list of equipment--except clothing--useful in the collection of fungi:

1. Vasculum or other container
2. Sharp pocket knife
3. Pruning shears
4. Small saw
5. Cold chisel and small hammer
6. Roll of wax paper
7. Pencil and note paper
8. Paper envelops for collecting soil samples
9. Various jars for collecting aquatics
10. Plant press with newspapers, blotters, and corrugated sheets
11. Hand lens
12. Mosquito repellent.

B. PRESERVATION OF FUNGI

1. General preservative for fungi. It is desirable to have some of the seasonal fungi preserved for study during various times of the year. For fungi

such as mushrooms or for diseased plant material a general preservative containing 5 parts of 40% formaldehyde in 95 parts of water will keep the material for an indefinite period of time. Another commonly used fixative contains the following ingredients:

Formaldehyde (40%)	50 ml.
Glacial acetic acid	50 ml.
Alcohol (50 or 70%)	900 ml.

2. Preservatives for colored fungi. Specimens must be retained in the solutions.

   a. Color not water soluble:

Mercuric acetate	10 gm.
Glacial acetic acid	5 ml.
Water	1000 ml.

   b. Color soluble in water:

Mercuric acetate	1 gm.
Neutral lead acetate	10 gm.
Glacial acetic acid	10 ml.
Alcohol (90 per cent)	1000 ml.

   c. Zinc sulphate preservative:

Zinc sulphate	25 gm.
Formaldehyde (40%)	10 ml.
Water	1000 ml.

3. Preservative for green plants with parasitic fungi. It is frequently desirable to preserve the green color of the host plant. This can be done by placing the fresh specimens in a boiling mixture containing 1 part of glacial acetic acid saturated with copper acetate crystals and 4 parts of water. The boiling is continued until the copper acetate replaces the broken down chlorophyll in the host cells. The treated specimens should be kept in 5% formalin.

   Another good method is to place the materials in a 5% solution of copper sulphate for at least 6 hours or not more than 24 hours. Wash in running water for several hours, and place in a preserving liquid (containing 5-6% SO_2 solution, 15 ml; and 1000 ml. of distilled water) in a sealed container. For fruits, add 20 to 30 ml. of white glycerine to the preservative (Goncalves, 1931).

C. MAINTENANCE OF STOCK CULTURES OF FUNGI. Most fungi can be kept alive on many of the agar media for 6 months or 1 year before transfers are necessary. Sporulating agar cultures may be frozen at - 20° C. in the deep freeze and maintained for considerable periods of time (Carmichael, 1956). Another good method for keeping cultures for a number of years is by pouring sterilized mineral oil in the test tube until the culture is completely submerged (Wernham, 1946). The best method for preservation of most fungi that sporulate is by the lyophil process or the freeze dry method (Raper and Alexander, 1945). Cultures have been maintained for at least 20 years by this method.

D. PRESERVATION BY DRYING. Most fungi can be preserved indefinitely by drying, but this method is more satisfactory for some than it is for others. Fungi

parasitic on leaves and herbaceous stems are generally dried in a plant press, as mentioned in a previous paragraph. In a dry, hot climate it is generally sufficient to place the press containing the specimens in the sun for two or three days in order for drying to be complete. In a moist climate the blotters need to be changed every day otherwise the specimens are very likely to mold. Under any circumstances where speed in drying is essential the press may be stood edgewise over a heating devise to dry the specimens quickly by permitting the hot air to circulate through the corrugated sheets used between the blotters. After the specimens are dried they are removed from the press and placed in envelops on which the pertinent data are written.

Dryers for fleshy specimens may be made easily by fitting a metal, wooden, or asbestos box with sockets bearing electric light bulbs, and covering the box with a metal screen on which the specimens to be dried are placed. After drying, the specimens should be stored in convenient-sized, heavy pasteboard boxes. Some insect fumigant such as paradichlorobenzene crystals should be kept in each box containing dried specimens to prevent destruction by insects.

VI. Sources of Supplies for Mycology Courses

A. The following is a list of some of the commercial companies where supplies may be procured for courses in mycology:

1. Laboratory supplies:

Aloe Scientific Co., 5655 Kingsbury, St. Louis 12, Mo.
Arthur H. Thomas Co., West Washington Square, Philadelphia, Pa.
Carolina Biological Supply Co., Elon College, North Carolina.
Central Scientific, 1700 Irving Park Road, Chicago 13, Ill.
Chicago Apparatus Co., 1735 North Ashland Ave., Chicago 22, Ill.
Clay-Adams Co., Inc., 141 East 25th St., New York 10, N. Y.
Eberbach and Son Co., 200 East Liberty St., Ann Arbor, Mich.
E. H. Sargent and Co., 4647 W. Foster Ave., Chicago 30, Ill.
Eimer and Amend, 205 - 223 Third Ave., New York, N. Y.
Falcon Plastics, 6020 West Washington Boulevard, Culber City, Calif.
Fisher Scientific Co., 717 Forbes St., Pittsburg 19, Pa.
General Biochemicals, 690 Laboratory Park, Chagrin Falls, Ohio
General Biological Supply House (Turtox), 8200 South Hoyne Ave., Chicago 20, Ill.
Harshaw Scientific, 1945 East 97th St., Cleveland 6, Ohio
Schaar and Co., 754 W. Lexington St., Chicago 7, Illinois
Standard Scientific Supply Corporation, 808 Broadway, New York 3, N. Y.
The Chemical Rubber Co., 2310 Superior Ave., Cleveland 14, Ohio
Ward's Natural Science Establishment, Inc., 3000 Ridge Road East, Rochester 9, N. Y.
Will Corporation, Rochester 3, N. Y.
W. M. Welch Scientific Co., 1515 Sedgwick St., Chicago, Ill.

B. The concerns listed below specialize in the following items:

1. Cultures:

American Biological Culture House, Charleston, Ill.
American Type Culture Collection, 2029 M Street N. W., Washington 6, D. C.
Carolina Biological Supply Co., Elon College, North Carolina.
Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.
General Biological Supply House (Turtox), 8200 S. Hoyne Ave., Chicago 20, Ill.

2. Media:

Baltimore Biological Laboratory, Inc., 2201 Aisquith St., Baltimore 18, Md.
Ben Venue Laboratories, Inc., Bedford, Ohio (Media in tablet form. Excellent for preparing small quantitite of agar media.)
Difco Laboratories, Inc., Detroit 1, Michigan.

3. Slides:

Carolina Biological Supply Co., Elon College, North Carolina.
General Biological Supply House (Turtox), 8200 S. Hoyne Ave., Chicago 20, Ill.
Ripon Microslides Laboratory (John P. Limbach), Box 164, Ripon, Wis.
Triarch Botanical Products (George H. Conant), Ripon, Wis.

C. EDUCATIONAL FILMS ON FUNGI:

Life Cycle of a Yeast Cell. Black and White, 17 min. Southern Illinois University, Carbondale, Ill.
Life of the Molds. Color and black and white, 21 min. McGraw-Hill Text Films, 330 West 42nd St., New York 36, N. Y.
Miracle from Mold -- The Story of Terramycin. Chas. Pfizer & Co., 630 Flushing Ave., Brooklyn 6, N. Y.
Fungi. Color, 15 min. Encyclopaedia Brittanica Films, Inc.
Pin Mold. Black and white, 10 min. International Film Foundation.
Pythium aphanidermatum. Black and white, 28 min. Botany Dept., Univ. of Western Ontario, London, Canada.
Seifriz on Protoplasm. Black and white, 26 min. Educational Film Library Assoc., 345 East 46th St., New York 17, N. Y.
Slime Molds I: Life Cycle, U-5518. Color or Black and white, 30 min.
Slime Molds II: Collection, Cultivation, and Use, U-5519. Color, 19 min.
Slime Molds III: Identification, U-5520. Color, 24 min.
Bureau of Audio-visual Instruction, Extension Division, State University of Iowa, Iowa City, Iowa.
Syngamy and Alternation of Generations in Allomyces -- a Water Mold. Black and white, 20 min. Phase Films, Box 423, Ross, Calif.
The Biology of Atta, the Ants Which Grow Mushrooms. Service du Film de Recherche Scientifique.
The Story of Penicillin. Black and white, 10 min. Chas. Pfizer and Co., Inc., 630 Flushing Ave., Brooklyn 6, New York.

REFERENCES:

1. AINSWORTH, G. C., and G. R. BISBY. 1961. A dictionary of the fungi. viii plus 547 pp. The Imperial Mycological Institute, Kew, Surrey.
2. ALEXOPOULOS, C. J. 1936. A plant suitable for classroom demonstration of growth. Torreya 36:3-4.
3. BUELL, CAROLINE B., and W. H. WESTON. 1947. Application of the mineral oil conservation to maintaining collections of fungous cultures. Amer. Jr. Bot. 34:555-561.
4. BERLINER, M. D. 1961. Studies in fungal luminescence. Mycologia 53:84-90.
5. CARMICHAEL, J. W. 1956. Forzen storage for stock cultures of fungi. Mycologia 48:378-381.
6. CHRISTENEN, C. M. 1961. The molds and man. 2nd Ed. viii + 238 pp. University of Minnesota Press, Minneapolis, Minn.
7. EMERSON, R. 1958. Mycological organization. Mycologia 50:589-621.
8. FORBES, M. 1953. Materials and methods for the study of some common molds in high school biology. MS Thesis. Iowa State Teachers College. Cedar Falls, Iowa.
9. GOLDIE-SMITH, E. K. 1956. Maintenance of stock cultures of aquatic fungi. J. Elisha Mitch. Sci. Soc. 72:158.
10. GONCALVES, R. D. 1931. A liquid for the preservation of plant materials for museum purposes. Revista Agric. São Paolo, 6:315.

11. HEINLEIN, C. L. and COOKE, W. B. 1958. Micro-fungi in the soil. Sci. Teacher 25:20-24.
12. HESSELTINE, C. W., BARBARA J. BRADLE, and C. R. BENJAMIN. 1960. Further Investigations on the Preservation of Molds. Mycologia 52:762-773.
13. HOWARD, F. L. 1931. Laboratory cultivation of myxomycete plasmodia. Am. J. Bot. 18:624-628.
14. KONDO, W. T., S. O. GRAHAM, and C. G. SHAW. 1959. Modifications of the cellophane culture technique for photographing and preserving reference colonies of microorganisms. Mycologia 51:368-374.
15. LILLY, V. G., and H. L. BARNETT. 1951. Physiology of the fungi. xii, + 464 pp.
16. MACBRIDE, T. H., and G. W. MARTIN. 1934. The Myxomycetes. vii + 339 pp. The Macmillan Co., New York.
17. RAPER, K. B. 1951. Isolation, cultivation, and conservation of simple slime molds. The Quarterly Rev. Biology 26:169-190.
18. RAPER, K. B., and DOROTHY F. ALEXANDER. 1945. Preservation of molds by the lyophil process. Mycologia 37:499-525.
19. RAPER, K. B., AND THOM, C. 1945. A manual of the penicillia. ix + 373 pp. The Williams and Wilkins Co., Baltimore.
20. RAWLINS, T. E. 1933. Phytopathological and botanical research methods. John Wiley and Sons, Inc., New York.
21. RIDDELL, R. W. 1950. Permanent stained mycological preparations obtained by slide culture. Mycologia 42:265-270.
22. RIKER, A. J., and R. S. RIKER. 1936. Introduction to research on plant diseases. John S. Swift Co., St. Louis.
23. SLOAN, B. J., J. B. ROUTIEN, and V. P. MILLER. 1960 (1961). Increased sporulation in fungi. Mycologia 52:47-63.
24. SMITH, G. 1952. An introduction to industrial mycology. 4th Ed. 378 pp. Edward Arnold Publishers, Ltd. London.
25. SPARROW, F. K., JR. 1960. Aquatic phycomycetes. xvi + 1187 pp. Univ. Mich. Press. Ann Arbor.
26. THOM, C., and K. B. RAPER. 1945. ix + 373 pp. A manual of the Aspergilli. The Williams and Wilkins Co.
27. VANBREUSEGHEM, R. 1952. Technique biologique pour l'isolement des dermatophytes du sol. Ann. Soc. Belge Med. Trop., 32:173-178.
28. WERHANM, C. C. 1946. Mineral oil as a fungus culture preservative. Mycologia 38:691-692.

Part II

The Achlorophyllous Thallophytes (Slime Molds, Bacteria, and Fungi)

1. The traditional classification of all living organisms into two kingdoms, plant and animal, has been seriously questioned since Haeckel (1866) proposed the segregation of intermediate forms into the Protista. Some authors (Stanier, Doudoroff, and Adelberg, 1957) have accepted this third kingdom; others (Copeland, 1956; Ingold, 1961) have gone behond Haeckel and have recognized four or more kingdoms; and still others (Cronquist, 1960) prefer the status quo. We shall follow this most conservative view and shall place the bacteria and the fungi in the plant kingdom. In addition, we shall study the cellular slime molds, which are probably not related to either the bacteria or the fungi, and shall treat them separately, under the order Acrasiales, without attempting to place them in any Division or Kingdom.

2. Organisms in the plant kingdom which are of relatively simple structure, which have no stems, roots, or leaves, and which generally reproduce by spores are often referred to as thallophytes. In the older text books these organisms were all placed in the Division Thallophyta, but modern taxonomists no longer recognize this grouping. Instead, we now place these organisms into a large number of divisions. Some of these organisms possess chlorophyll; these are classified in the various divisions of algae. Others -- the achlorophyllous thallophytes -- do not have any chlorophyll; these we shall classify into two divisions, the Schizomycota (bacteria) and the Mycota (fungi, including the true slime molds).

The Slime Molds

3. The term slime mold has been used so loosely that few biologists, who have not worked with the fungi or protozoa, understand what organisms are included in this term. There are at least four distinct and probably unrelated or distantly related groups which have been called slime molds. These are the Acrasiales or cellular slime molds, the Labyrinthulales or net slime molds, the Plasmodiophoromycetes or endoparasitic slime molds, and the Myxomycetes or true slime molds. The last two groups belong to the fungi. The other two appear to be more closely related to the protozoa.

The Bacteria

4. All organisms we include under the term bacteria in its broadest meaning are placed in the Division (Phylum) Schizomycota which is subdivided into two classes, the Schizomycetes and the Microtatobiotes. The Schizomycetes are cellular. Their cells or filaments, which seldom exceed 2μ in diameter, psssess primitive nuclei devoid of nuclear membranes or nucleoli. Reproduction is usually by fission, but sexual or parasexual reproduction is known in some species and may be common throughout the group. The Microtatobiotes are at or beyond the limits of light microscope visibility. Their detailed structure and their life histories are, for the most part, unknown.

The Fungi

5. The fungi belong to the Division (Phylum) Mycota which includes two Sub-divisions (Sub-phyla): the Myxomycotina, consisting of the true slime molds, and the

the Eumycotina in which we place all the true fungi. The Mycota possess true nuclei with nuclear membranes and nucleoli. The soma varies from a microscopic single cell to an extensive plasmodium or mycelium. Reproduction, both sexual and asexual, results in the formation of spores which are the propagative units.

6. The partial outline which follows is not intended to be complete, but is rather designed to help you understand the relationships of the various organisms which are included in this manual and some others which are frequently discussed in an introductory course in mycology. For a more complete outline and for keys to the families and genera of the fungi consult the references at the end of the outline.

A Partial Outline of the Achlorophyllous Thallophytes

Organisms of Uncertain Affinities

Order Acrasiales (The Cellular Slime Molds)
- Family Acytosteliaceae
  - Genus *Acytostelium*
    - Example - *Acytostelium leptosomum*
  - Genus *Protostelium*
    - Example - *Protostelium mycophaga*
- Family Dictyosteliaceae
  - Genus *Dictyostelium*
    - Examples - *Dictyostelium mucoroides*
      Dictyostelium discoideum
  - Genus *Polysphondylium*
    - Example - *Polysphondylium violaceum*

Order Labyrinthulales (The Net Slime Molds)
- Family Labyrinthulaceae
  - Genus *Labyrinthula*
    - Example - *Labyrinthula macrocystis*

Division Schizomycota (The Bacteria)

Class Schizomycetes

Order Eubacteriales
- Examples - *Escherichia coli*
  Salmonella typhosa
  Erwinia amylovora
  Agrobacterium tumefaciens

Order Actinomycetales
- Family Mycobacteriaceae
  - Genus *Mycobacterium*
    - Examples - *Mycobacterium tuberculosis*
      Mycobacterium leprae
- Family Actinomycetaceae
  - Genus *Actinomyces*
    - Example - *Actinomyces bovis*
  - Genus *Nocardia*
    - Example - *Nocardia asteroides*
- Family Streptomycetaceae
  - Genus *Streptomyces*
    - Examples - *Streptomyces griseus*
      Streptomyces scabies
      Streptomyces venezuelae
- Family Actinoplanaceae
  - Genus *Actinoplanes*
    - Example - *Actinoplanes philippinensis*

Genus Streptosporangium
Example - Streptosporangium roseum

Class Microtatobiotes

Order Rickettsiales

Order Virales

Division Mycota (The Fungi)

Sub-division Myxomycotina (The True Slime Molds)

Class Myxomycetes

Sub-class Ceratiomyxomycetidae (Exosporeae)
Order Ceratiomyxales
Family Ceratiomyxaceae
Genus Ceratiomyxa
Example - Ceratiomyxa fruticulosa
Sub-class Myxogastromycetidae (Myxogastres)
Order Physarales
Family Physaraceae
Genus Physarum
Example - Physarum polycephalum
Genus Badhamia
Example - Badhamia folliicola
Genus Fuligo
Example - Fuligo septica
Family Didymiaceae
Genus Didymium
Example - Didymium melanospermum
Genus Diderma
Example - Diderma testaceum
Order Stemonitales
Family Stemonitaceae
Genus Stemonitis
Example - Stemonitis fusca
Genus Comatricha
Example - Comatricha typhoides
Order Echinosteliales
Family Echinosteliaceae
Genus Echinostelium
Example - Echinostelium minutum
Order Liceales
Family Reticulariaceae
Genus Lycogala
Example - Lycogala epidendrum

Order Trichiales
Family Trichiaceae
Genus Trichia
Example - Trichia scabra
Genus Hemitrichia
Example - Hemitrichia clavata
Genus Acryria
Example - Acryria denudata

Sub-division Eumycotina

Class Chytridiomycetes

Order Chytridiales
Family Olpidiaceae
Genus Olpidium
Examples - Olpidium brassicae
Olpidium viciae
Genus Rozella
Example - Rozella achlyae
Family Synchytriaceae
Genus Synchytrium
Example - Synchytrium endobioticum
Family Phlyctidiaceae
Genus Rhizophidium
Example - Rhizophidium couchii
Family Cladochytriaceae
Genus Physoderma
Example - Physoderma zea-maydis
Family Chytridiaceae
Genus Chytridium
Example - Chytridium olla
Family Megachytriaceae
Genus Nowakowskiella
Example - Nowakowskiella elegans
Order Blastocladiales
Family Blastocladiaceae
Genus Blastocladia
Example - Blastocladia Pringsheimii
Genus Allomyces
Example - Allomyces javanîcus
Order Monoblepharidales
Family Monoblepharidaceae
Genus Monoblepharis
Example - Monoblepharis polymorpha
Genus Monoblepharella
Example - Monoblepharella taylori
Genus Gonapodya
Example - Gonapodya prolifera

Class Hyphochytridiomycetes

Order Hyphochytriales
- Family Rhizidiomycetaceae
  - Genus _Rhizidiomyces_
    - Example - _Rhizidiomyces apophysatus_
- Family Anisolpidiaceae
- Family Hyphochytriaceae

Class Oömycetes

Order Lagenidiales
- Family Olpidiopsidaceae
  - Genus _Olpidiopsis_
    - Example - _Olpidiopsis varians_
- Family Lagenidiaceae
  - Genus _Lagenidium_
    - Example - _Lagenidium rabenhorstii_

Order Saprolegniales
- Family Ectrogellaceae
  - Genus _Ectrogella_
- Family Thraustochytriaceae
  - Genus _Thraustochytrium_
- Family Saprolegniaceae
  - Genus _Saprolegnia_
    - Example - _Saprolegnia ferax_
  - Genus _Achlya_
    - Example - _Achlya ambisexualis_
  - Genus _Dictyuchus_
    - Example - _Dictyuchus monosporus_
  - Genus _Thraustotheca_
    - Example - _Thraustotheca primoachlya_
  - Genus _Geolegnia_
    - Example - _Geolegnia inflata_

Order Leptomitales
- Family Leptomitaceae
  - Genus _Apodachlya_
    - Example - _Apodachlya pyrifera_
  - Genus _Leptomitus lacteus_
    - Example - _Leptomitus_
- Family Rhipidiaceae
  - Genus _Araiospora_
    - _Mindeniella_
    - _Rhipidium_
    - _Sapromyces_

Order Peronosporales
- Family Pythiaceae
  - Genus _Zoöphagus_
    - Example - _Zoöphagus insidians_
  - Genus _Pythium_
    - Examples - _Pythium aphanidermatum_
    - _Pythium debaryanum_
  - Genus _Phytophthora_
    - Examples - _Phytophthora cactorum_
    - _Phytophthora fragariae_
    - _Phytophthora infestans_

Family Peronosporaceae
Genus Plasmopara
Example - Plasmopara viticola
Genus Peronospora
Example - Peronospora parasitica
Genus Basidiophora
Example - Basidiophora entospora
Genus Sclerospora
Example - Sclerospora graminicola
Genus Bremia
Example - Bremia lactucae
Genus Pseudoperonospora
Example - Pseudoperonospora cubensis
Family Albuginaceae
Genus Albugo
Examples - Albugo candida
Albugo bliti
Albugo tragopogonis

Class Plasmodiophoromycetes

Order Plasmodiophorales
Family Plasmodiophoraceae
Genus Plasmodiophora
Example - Plasmodiophora brassicae
Genus Spongospora
Example - Spongospora subterranea

Class Zygomycetes

Order Mucorales
Family Mucoraceae
Genus Rhizopus
Example - Rhizopus stolonifer
Genus Mucor
Example - Mucor genevensis
Genus Phycomyces
Example - Phycomyces blakesleanus
Genus Absidia
Example -Absidia glauca
Genus Zygorhynchus
Example - Zygorhynchus heterogamus
Family Thamnidiaceae
Genus Helicostylum
Example - Helicostylum elegans
Family Cunninghamellaceae
Genus Cunninghamella
Example - Cunninghamella echinulata
Family Choanephoraceae
Genus Choanephora
Example - Choanephora cucurbitarum
Genus Blakeslea
Example - Blakeslea trispora

Family Pilobolaceae
    Genus Pilobolus
        Example - Pilobolus crystallinus
Family Mortierellaceae
    Genus Mortierella
        Example - Mortierella candelabrum
Family Endogonaceae
    Genus Endogone
        Example - Endogone sphagnophila
Family Syncephalastraceae
    Genus Syncephalastrum
        Example - Syncephalastrum racemosum
Family Piptocephalidaceae
    Genus Piptocephalis
        Example - Piptocephalis freseniana
    Genus Syncephalis
        Example - Syncephalis cordata
Family Dimargaritaceae
    Genus Dimargaris
        Dispira
        Spinalia
Family Kickxellaceae
    Genus Coemansia
        Kickxella
        Spirodactylon
Order Entomophthorales
    Family Entomophthoraceae
        Genus Entomophthora
            Example - Entomophthora muscae
    Family Basidiobolaceae
        Genus Basidiobolus
            Example - Basidiobolus ranarum
        Genus Conidiobolus
            Example - Conidiobolus brefeldianus
Order Zoöpagales
    Family Zoöpagaceae
        Genus Endocochlus
            Cochlonema
            Zoöpage
            Stylopage
            Cystopage

Class Trichomycetes

Order Eccrinales
    Family Eccrinaceae
        Genus Enterobryus
            Example - Enterobryus borariae
Order Amoebidiales
    Harpelales
    Asellariales
    Genistalales

Class Ascomycetes

Sub-class Hemiascomycetidae
Order Endomycetales
Family Ascoideaceae
Genus Dipodascus
Example - Dipodascus uninucleatus
Genus Ascocybe
Example - Ascocybe grovesii
Genus Endomycopsis
Example - Endomycopsis fibuliger
Genus Eremascus
Example - Eremascus fertilis
Family Saccharomycetaceae
Genus Schizosaccharomyces
Example - Schizosaccharomyces octosporus
Genus Saccharomyces
Example - Saccharomyces cerevisiae
Genus Hansenula
Example - Hansenula saturnus
Family Spermophthoraceae
Genus Nematospora
Example - Nematospora coryli
Order Taphrinales
Family Taphrinaceae
Genus Taphrina
Examples - Taphrina deformans
Taphrina cerasi
Taphrina pruni
Sub-class Euascomycetidae
Series Plectomycetes
Order Eurotiales
Family Ascosphaeriaceae;
Genus Ascosphaera
Example - Ascosphaera apis
Family Gymnoascaceae
Genus Myxotrichum
Example - Myxotrichum uncinatum
Genus Ctenomyces
Example - Ctenomyces serratus
Genus Arthroderma
Example - Arthroderma quadrifidum
Genus Nannizzia
Example - Nannizzia obtusa
Family Eurotiaceae
Genus Eurotium
Example - Eurotium repens
Genus Emericella
Example - Emericella nidulans
Genus Sartorya
Example - Sartorya fumigata
Genus Carpenteles
Example - Carpenteles javanicum
Tenus Tallaromyces
Example - Tallaromyces vermiculatus

- Order Onygenales
  - Family Onygenaceae
    - Genus *Onygena*
      - Example - *Onygena equina*
  - Family Trichocomaceae
  - Family Dendrosphaeriaceae
- Order Microascales
  - Family Microascaceae
    - Genus *Microascus*
  - Family Ophiostomataceae
    - Genus *Ceratocystis*
      - Examples - *Ceratocystis ulmi*
        Ceratocystis fagacearum

Series Pyrenomycetes

- Order Erysiphales
  - Family Erysiphaceae
    - Genus *Erysiphe*
      - Example - *Erysiphe graminis*
    - Genus *Sphaerotheca*
      - Example - *Sphaerotheca pannosa*
    - Genus *Microsphaera*
      - Example - *Microsphaera alni*
    - Genus *Podosphaera*
      - Example - *Podosphaera oxyacanthae*
    - Genus *Uncinula*
      - Example - *Uncinula necator*
    - Genus *Phyllactinia*
      - Example - *Phyllactinia corylea*
- Order Meliolales
  - Family Meliolaceae
    - Genus *Meliola*
      Irene
- Order Chaetomiales
  - Family Chaetomiaceae
    - Genus *Chaetomium*
      - Example - *Chaetomium globosum*
- Order Sphaeriales
  - Family Sordariaceae
    - Genus *Sordaria*
      - Example - *Sordaria fimicola*
    - Genus *Podospora*
      - Example - *Podospora anserina*
    - Genus *Neurospora*
      - Example - *Neurospora sitophila*
    - Genus *Gelasinospora*
      - Examples - *Gelasinospora cerealis*
        Gelasinospora calospora
        Gelasinospora tetrasperma
  - Family Phyllachoraceae
    - Genus *Phyllachora*
      - Example - *Phyllachora graminis*
  - Family Diatrypaceae
    - Genus *Diatrype*
      - Example - *Diatrype disciformis*
  - Family Xylariaceae
    - Genus *Rosellinia*
      - Example - *Rosellinia ligniaria*

Genus Xylaria
Example - Xylaria polymorpha
Genus Nummularia
Example - Nummularia discreta
Genus Daldinia
Example - Daldinia concentrica
Order Diaporthales
Family Gnomoniaceae
Genus Gnomonia
Example - Gnomonia ulmea
Family Diaporthaceae
Genus Diaporthe
Example - Diaporthe citri
Genus Endothia
Example - Endothia parasitica
Genus Glomerella
Example - Glomerella cingulata
Order Hypocreales
Family Nectriaceae
Genus Nectria
Example - Nectria cinnabarina
Family Hopocreaceae
Genus Hypocrea
Example - Hypocrea sulphurea
Family Hypomycetaceae
Genus Hypomyces
Example - Hypomyces hyalinus
Family Melanosporaceae
Genus Melanospora
Example - Melanospora chionea
Order Clavicipitales
Family Clavicipitaceae
Genus Claviceps
Example - Claviceps purpurea
Genus Cordyceps
Example - Cordyceps militaris
Order Coryneliales
Order Coronophorales
Series Discomycetes
Order Ostropales
Order Helotiales
Family Sclerotiniaceae
Genus Monilinia
Example - Monilinia fructicola
Genus Stromatinia
Example - Stromatinia gladioli
Genus Sclerotinia
Example - Sclerotinia sclerotiorum
Family Phacidiaceae
Genus Rhytisma
Example - Rhytisma acerinum
Genus Lophodermium
Example - Lophodermium pinastri
Family Geoglossaceae
Genus Geoglossum
Example - Geoglossum ophioglossoides

Genus _Spathularia_
Example - _Spathularia_ _clavata_
Family Cyttariaceae
Genus _Cyttaria_
Example - _Cyttaria_ _gunnii_
Order Pezizales
Family Sarcoscyphaceae
Genus _Sarcoscypha_
Example - _Sarcoscypha_ _coccinea_
Genus _Urnula_
Example - _Urnula_ _craterium_
Family Pezizaceae
Genus _Peziza_
Example - _Peziza_ _badio-confusa_
Genus _Ascobolus_
Example - _Ascobolus_ _magnificus_
Family Helvellaceae
Genus _Helvella_
Example - _Helvella_ _esculenta_
Genus _Morchella_
Example - _Morchella_ _conica_
Genus _Verpa_
Example - _Verpa_ _bispora_
Order Tuberales
Family Tuberaceae
Genus _Tuber_
Example - _Tuber_ _aestivum_
Family Elaphomycetaceae
Genus _Elaphomyces_
Series Laboulbeniomycetes
Order Laboulbeniales
Family Laboulbeniaceae
Genus _Laboulbenia_
Example - _Laboulbenia_ _formicarum_
Sub-class Loculoascomycetidae
Order Myriangiales
Family Elsinoeaceae
Genus _Elsinoe_
Examples - _Elsinoe_ _ampelina_
Elsinoe _fawcettii_
Elsinoe _veneta_
Family Myriangiaceae
Genus _Myriangium_
Example - _Myriangium_ _bambusae_
Family Piedraiaceae
Genus _Piedraia_
Example - _Piedraia_ _hortai_
Order Dothideales
Family Dothideaceae
Genus _Dothidea_
Example - _Dothidea_ _collecta_
Genus _Mycosphaerella_
Examples - _Mycosphaerella_ _musicola_
Mycosphaerella _fragariae_
Genus _Guignardia_
Example - _Guignardia_ _bidwellii_

Family Pseudosphaeriaceae
Genus _Pseudoplea_
Example - _Pseudoplea gäumannii_
Family Capnodiaceae
Genus _Capnodium_
Example - _Capnodium citri_
Order Pleosporales
Family Pleosporaceae
Genus _Pleospora_
Example - _Pleospora herbarum_
Family Venturiaceae
Genus _Venturia_
Example - _Venturia inaequalis_
Family Lophiostomataceae
Order Microthyriales
Order Hysteriales
Family Hysteriaceae
Genus _Hysterium_
Example - _Hysterium insidens_
Genus _Glonium_
Example - _Glonium stellatum_

Form-Class Deuteromycetes (Fungi Imperfecti)

Form-Order Sphaeropsidales
Form-Family Sphaeropsidaceae
Section Amerosporae
Sub-section Hyalosporae
Form-Genus _Phyllosticta_
Example - _Phyllosticta solitaria_
Form-Genus _Phoma_
Example - _Phoma betae_
Form-Genus _Dendrophoma_
Example - _Dendrophoma obscurans_
Sub-section Phaeosporae
Form-Genus _Sphaeropsis_
Example - _Sphaeropsis malorum_
Form-Genus _Coniothyrium_
Example - _Coniothyrium ribis_
Section Didymosporae
Sub-section Hyalodidymae
Form-Genus _Ascochyta_
Example - _Ascochyta pisi_
Sub-section Phaeodidymae
Form-Genus _Diplodia_
Example - _Diplodia zeae_
Section Scolecosporae
Form-Genus _Septoria_
Example - _Septoria apii_
Form-Family Zythiaceae
Section Amerosporae
Sub-section Hyalosporae
Form-Genus _Zythia_
Example - _Zythia fragariae_
Form-Family Leptostromataceae
Form-Family Excipulaceae

Form-Order Melanconiales
- Form-Family Melanconiaceae
  - Section Amerosporae
    - Sub-section Hyalosporae
      - Form-Genus Gloeosporium
        - Example - Gloeosporium perennans
      - Form-Genus Colletotrichum
        - Example - Colletotrichum lindemuthianum
  - Section Didymosporae
    - Sub-section Hyalodidymae
      - Form-Genus Marssonina
        - Examples - Marssonina juglandis
          Marssonina rosae
          Marssonina fragariae
  - Section Phragmosporae
    - Sub-section Hyalophragmiae
      - Form-Genus Entomosporium
        - Example - Entomosporium maculatum
    - Sub-section Phaeophragmiae
      - Form-Genus Pestalotia
        - Example - Pestalotia guepinii
  - Section Scolecosporae
    - Form-Genus Cylindrosporium
      - Example - Cylindrosporium pomi

Form-Order Moniliales
- Form-Family Moniliaceae
  - Section Amerosporae
    - Form-Genus Botrytis
      - Example - Botrytis tulipae
    - Form-Genus Geotrichum
      - Example - Geotrichum candidum
    - Form-Genus Verticillium
      - Example - Verticillium albo-atrum
  - Section Didymosporae
    - Form-Genus Trichothecium
      - Example - Trichothecium roseum
  - Section Phragmosporae
    - Form-Genus Epidermophyton
      - Example - Epidermophyton floccosum
- Form-Family Dematiaceae
  - Section Amerosporae
    - Form-Genus Hormodendrum
      - Example - Hormodendrum pedrosoi
  - Section Didymosporae
    - Form-Genus Fusicladium
      - Example - Fusicladium carpophilum
  - Section Phragmosporae
    - Form-Genus Helminthosporium
      - Example - Helminthosporium teres
    - Form-Genus Heterosporium
      - Example - Heterosporium iridis
  - Section Dictyosporae
    - Form-Genus Alternaria
      - Example - Alternaria solani
    - Form-Genus Stemphylium
      - Example - Stemphylium botryosum

Section Scolecosporae
Form-Genus Cercospora
Example - Cercospora apii
Section Helicosporae
Form-Genus Helicosporium
Example - Helicosporium vegetum
Form-Family Tuberculariaceae
Section Amerosporae
Sub-section Hyalosporae
Form-Genus Volutella
Example - Volutella fructi
Form-Genus Tubercularia
Example - Tubercularia vulgaris
Sub-section Phaeosporae
Form-Genus Epicoccum
Example - Epicoccum nigrum
Section Phragmosporae
Sub-section Phaeophragmiae
Form-Genus Exosporium
Example - Exosporium melampsoroides
Form-Family Stilbellaceae
Section Hyalostilbeae-Amerosporae
Form-Genus Isaria
Section Phaeostilbeae-Amerosporae
Form-Genus Coremium
Example - Coremium glaucum
Form-Genus Graphium
Example - Graphium ulmi
Form-Family Cryptococcaceae
Form-Genus Cryptococcus
Example - Cryptococcus neoformans
Form-Genus Candida
Example - Candida albicans
Form-Genus Trichosporon
Example - Trichosporon beigeli
Form-Order Mycelia Sterilia
Form-Genus Rhizoctonia
Example - Rhizoctonia solani
Form-Genus Sclerotium
Example - Sclerotium rolfsii

Class Basidiomycetes

Sub-class Heterobasidiomycetidae
Order Tremellales
Family Ceratobasidiaceae
Genus Ceratobasidium
Metabourdotia
Family Dacrymycetaceae
Genus Dacrymyces
Example - Dacrymyces deliquescens
Genus Calocera
Example - Calocera cornea
Family Tremellaceae
Genus Exidia
Example - Exidia spiculosa

- Genus *Tremella*
  - Example - *Tremella fuciformis*
- Genus *Phlogiotis*
  - Example - *Phlogiotis helvelloides*

- Family Auriculariaceae
  - Genus *Auricularia*
    - Example - *Auricularia auricularis*
  - Genus *Herpobasidium*
    - Example - *Herpobasidium deformans*
- Family Phleogenaceae
  - Genus *Phleogena*
    - Example - *Phleogena faginea*
- Family Sporobolomycetaceae
  - Genus *Sporobolomyces*
    - *Sporidiobolus*
    - *Bullera*
    - *Itersonilia*
    - *Tilletiopsis*

- Order Uredinales
  - Family Pucciniaceae
    - Genus *Puccinia*
      - Example - *Puccinia graminis*
    - Genus *Uromyces*
      - Example - *Uromyces appendiculatus*
    - Genus *Gymnosporangium*
      - Example - *Gymnosporangium juniperi-virginianae*
    - Genus *Phragmidium*
      - Example - *Phragmidium rubi*
  - Family Melampsoraceae
    - Genus *Melampsora*
      - Example - *Melampsora chionea*
    - Genus *Cronartium*
      - Example - *Cronartium ribicola*
  - Family Coleosporiaceae
    - Genus *Coleosporium*
      - Example - *Coleosporium solidaginis*
- Order Ustilaginales
  - Family Ustilaginaceae
    - Genus *Ustilago*
      - Example - *Ustilago maydis*
    - Genus *Sorosporium*
      - Example - *Sorosporium reilianum*
    - Genus *Sphacelotheca*
      - Example - *Sphacelotheca sorghi*
    - Genus *Cintractia*
      - Example - *Cintractia axicola*
  - Family Tilletiaceae
    - Genus *Tilletia*
      - Example - *Tilletia carries*
    - Genus *Entyloma*
      - Example - *Entyloma calendulae*
  - Family Graphiolaceae
    - Genus *Graphiola*
      - Example - *Graphiola phoenicis*

- Sub-class Homobasidiomycetidae
  - Order Exobasidiales
    - Family Exobasidiaceae
      - Genus *Exobasidium*
        - Example - *Exobasidium vaccinii*
  - Series Hymenomycetes
    - Order Polyporales
      - Family Thelephoraceae
        - Genus *Stereum*
          - Example - *Stereum frustulosum*
        - Genus *Pellicularia*
          - Example - *Pellicularia filamentosa*
      - Family Clavariaceae
        - Genus *Clavaria*
          - Example - *Clavaria subbotrytis*
      - Family Cantharellaceae
        - Genus *Cantharellus*
          - Example - *Cantharellus cibarius*
      - Family Hydnaceae
        - Genus *Hericium*
          - Example - *Hericium coralloides*
      - Family Polyporaceae
        - Genus *Polyporus*
          - Example - *Polyporus cinnabarinus*
        - Genus *Fomes*
          - Example - *Fomes applanatus*
      - Family Meruliaceae
        - Genus *Merulius*
          - Example - *Merulius lacrymans*
    - Order Agaricales
      - Family Boletaceae
        - Genus *Boletus*
          - Example - *Boletus edulis*
        - Genus *Boletinus*
          - Example - *Boletinus porosus*
      - Family Paxillaceae
        - Genus *Paxillus*
          - Example - *Paxillus involutus*
      - Family Russulaceae
        - Genus *Russula*
          - Example - *Russula emetica*
        - Genus *Lactarius*
          - Example - *Lactarius piperatus*
      - Family Hygrophoraceae
        - Genus *Hygrophorus*
          - Example - *Hygrophorus conicus*
      - Family Agaricaceae
        - Genus *Amanita*
          - Example - *Amanita muscaria*
        - Genus *Pleurotus*
          - Example - *Pleurotus sapidus*
        - Genus *Pluteus*
          - Example - *Pluteus cervinus*
        - Genus *Pholiota*
          - Example - *Pholiota praecox*

Genus Agaricus
Example - Agaricus campestris
Genus Coprinus
Example - Coprinus comatus
Series Gasteromycetes
Order Lycoperdales
Family Lycoperdaceae
Genus Lycoperdon
Example - Lycoperdon pyriforme
Genus Calvatia
Example - Calvatia gigantea
Family Geastraceae
Genus Geastrum
Example - Geastrum rufescens
Order Sclerodermatales
Family Sclerodermataceae
Genus Scleroderma
Example - Scleroderma aurantium
Order Phallales
Family Phallaceae
Genus Phallus
Example - Phallus impudicus
Genus Dictyophora
Example - Dictyophora duplicata
Order Nidulariales
Family Nidulariaceae
Genus Crucibulum
Example - Crucibulum vulgare
Genus Cyathus
Example - Cyathus stercoreus
Family Sphaerobolaceae
Genus Sphaerobolus
Example - Sphaerobolus iowensis

REFERENCES:

1. AINSWORTH, G. C. 1961. A dictionary of the fungi. viii + 547 pp. The Imperial Mycological Institute, Kew, Surrey.
2. CLEMENTS, F. E., and CORNELIUS L. SHEAR. 1931. The genera of the fungi. iv + 496 pp. H. W. Wilson Company. New York.
3. COPELAND, H. F. 1956. The classification of lower organisms. ix - 302 pp. 45 figs. Frontisp. Pacific Books, Palo Alto.
4. CRONQUIST, A. 1960. The divisions and classes of plants. Bot. Rev., 26:425-482.
5. ENGLER, A. und K. PRANTL. 1897, 1900. Die natürlichen Pflazenfamilien. I. Teil, Abteil 1:1-513. I. Teil, Abteil. 1**:1-570.
6. HAECKEL, E. H. 1866. Generelle Morphologie. 2 vols. Berlin.
7. INGOLD, C. T. 1961. The Biology of Fungi. 124 pp. 61 figs. Hutchinson Educational Ltd., London.
8. MARTIN, G. W. 1950. Outline of the fungi. Wm. C. Brown Company, Dubuque, Iowa.
9. SACCARDO, P. A. 1882-1931. Sylloge fungorum omnium hucusque cognitorum. Vol. 1-25. Published by the author, Pavia, Italy.
10. STANIER, R. Y., M. DOUDOROFF, and E. A. ADELBERG. 1957. The microbial world. xxii - 682 pp. Prentice-Hall, Inc., Englewood Cliffs, N. J.

Organisms of Uncertain Affinity– The Cellular Slime Molds

A. GENERAL REMARKS:

1. The Cellular Slime Molds, all classified in the Order Acrasiales, are probably protozoa related to the amoebae. They are included here only because they are traditionally studied by mycologists.

2. The somatic structure of the Acrasiales is a uninucleate amoeba. This is an independent unit which feeds on bacteria and which divides repeatedly by fission thus producing a population of amoebae.

3. When a certain population density has been reached, aggregation centers are set up and the amoebae, orienting themselves toward the center, stream toward it and eventually coalesce forming a pseudoplasmodium. They do not, however, actually fuse, but retain their individuality and can be separated from each other by mechanical means. Nevertheless, the pseudoplasmodium behaves as a unit.

4. In some species the pseudoplasmodium migrates for sometime before it heaps itself up and differentiates into a fructification, the sorocarp. The mature sorocarp consists of a stalk, which in most species is cellular, and of a mass of spores at its tip. In the majority of species each spore has a wall of its own, but in a few forms the spores are naked and are then called pseudospores. The entire head of the sorocarp is enveloped in a drop of mucus. There is no peridium enclosing the spores.

5. Whether the Acrasiales reproduce sexually is a controversial issue. The fact that certain strains are known to be haploid and others, of the same species, to be diploid, argues for a sexual cycle. Sexual fusions of amoebae and the occurrence of meiosis in zygote cells have been reported by Wilson (1953) and by Wilson and Ross (1957).

6. The Acrasiales are subdivided into four families: Sappiniaceae, Guttulinaceae, Acytosteliaceae, and Dictyosteliaceae.

B. PROCEDURE:

Order: Acrasiales

7. Collect various samples of soil and of leaf mold in sterile containers. Make suspensions in sterile water and streak the surface of glucose-peptone or hay infusion agar plates, making several parallel streaks about 1/2 inch apart. Streak a suspension of Escherichia coli or Aerobacter aerogenes crosswise several times so as to form a checkerboard with the soil sample streaks. Incubate at room temperature. Examine for the presence of pseudoplasmodia or sorocarps a few days later. These are most likely to occur at the intersection of the streaks.

8. The cultures will undoubtedly be contaminated with fungi. To obtain two-membered cultures, touch a mature spore mass with a sterile needle or loop and transfer spores to a new Petri dish with glucose-peptone agar. Add a drop or two of bacterial suspension.

Family: Dictyosteliaceae

Genera: Dictyostelium, Polysphondylium

9. Examine a growing culture of Dictyostelium discoideum. Mount some myxamoebae in aceto-carmine. This should stain the nuclei.

10. Observe a culture in which pseudoplasmodia have been formed. Note the structure of this phase. Observe at intervals and note the migration of the pseudoplasmodia. This is a characteristic of this species.

11. Cut out a piece of agar bearing a pseudoplasmodium and immerse it in a dish of distilled water under the dissecting microscope. Observe any changes which take place.

12. Examine a fruiting culture of the same organism. Mount a sorocarp and observe under the microscope. Study the structure of the stalk; the spores.

13. Examine a fruiting culture of Polysphondylium violaceum. Note the whorled arrangement of the sorocarp branches. Note the color of the spore masses.

C. QUESTIONS:

1. Characterize the Acrasiales.
2. Construct a dichotomous key to the families and genera in this order.
3. What is acrasin? What role does it play in the life cycle of the Acrasiales?
4. What changes take place in the amoebae during aggregation?
5. Discuss three prominent theories concerning the initiation of aggregation (Wilson, 1953; Ennis and Sussman, 1958; Konijn and Raper, 1961).
6. What evidence do we have for sexual reproduction in the Acrasiales? What is the evidence against sexual reproduction?
7. Construct a life-cycle diagram of Dictyostelium discoideum.

D. TEXT REFERENCES:

Alexopoulos -- pp. 45-57.
Bessey -- pp. 29-30.

E. SELECTED REFERENCES:

1. BONNER, J. T. 1959. The cellular slime molds. x + 150 pp. 13 figs. 8 pls. Princeton University Press, Princeton.
2. BONNER, J. T. 1960. Development of the cellular slime molds: The role of cell division, cell size, and cell number. In:. Developing cell systems and their control. Soc. Study Devel. and Growth. 18th Growth Symposium. The Ronald Press Company, New York.
3. ENNIS, H. L. and M. SUSSMAN. 1958. The initiator cell for slime mold aggregation. Proc. Nat. Acad. Sci., 44:401-411.
4. KONIJN, T. M. and K. B. RAPER. 1961. Cell aggregation in Dictyostelium discoideum. Devel. Biol., 3:725-756.
5. OLIVE, E. W. 1902. Monograph of the Acrasieae. Proc. Boston Soc. Nat. Hist., 30:451-515.
6. RAPER, K. B. 1935. Dictyostelium discoideum a new species of slime mold from decaying forest leaves. Jour. Agr. Res., 50:135-147.
7. RAPER, K. B. 1951. Isolation, cultivation and conservation of simple slime molds. Quart. Rev. Biol., 26:169-190.
8. RAPER, K. B. 1960. Levels of cellular interaction in amoeboid populations. Proc. Am. Phil. Soc., 104:579-604.
9. RAPER, K. B. and DOROTHY I. FENNELL. 1952. Stalk formation in Dictyostelium. Bull. Torrey Bot. Club, 79:25-51.
10. RAPER, K. B. and CHARLES THOM. 1941. Interspecific mixtures in the Dictyosteliaceae. Am. Jr. Bot., 28:69-78.
11. ROSS, I. K. 1960. Studies on diploid strains of Dictyostelium discoideum. Am. Jr. Bot., 47:54-59.
12. SUSSMAN, R. R., M. SUSSMAN, and H. L. ENNIS. 1960. The appearance and inheritance of the I-cell phenotype in Dictyostelium discoideum. Devel. Biol., 2:367-392.
13. WILSON, C. M. 1953. Cytological study of the life cycle of Dictyostelium. Am. Jr. Bot., 40:714-718.
14. WILSON, C. M. and I. K. ROSS. 1957. Further cytological studies in the Acrasiales. Am. Jr. Bot., 44:345-350.

F. GLOSSARY:

1. Fructification (L. fructus = fruit): any fungal structure which contains spores.
2. Peridium (p. peridia; Gr. peridion = small pouch): the outside covering or wall of a fructification.
3. Pseudoplasmodium (pl. pseudoplasmodia; Gr. pseudo = false + plasmodium): an aggregation of amoeboid cells constituting the initial phase of fruiting in the Acrasiales.
4. Pseudospore (Gr. pseudo = false + sporos = seed, spore): a spore-like protoplast without a cell wall.
5. Sorocarp (Gr. soros = heap + karpos = fruit): the fructification of the Acrasiales.
6. Spore (Gr. sporos = seed, spore): a minute propagative unit functioning as a seed, but differing from a seed in that it does not contain a preformed embryo.

ORDER ACRASIALES

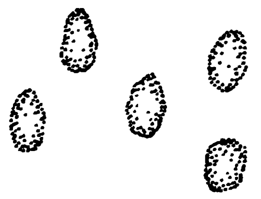


FIG. 1 AMOEBAE

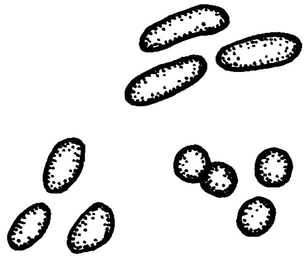


FIG. 2 SPORES

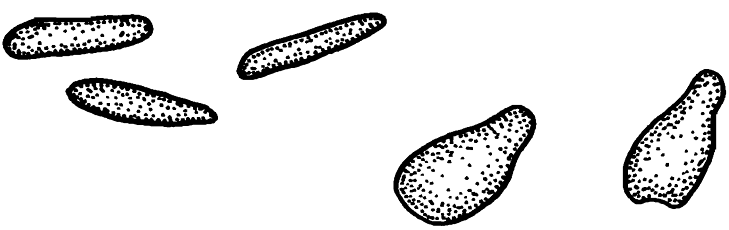


FIG. 3 PSEUDOPLASMODIA

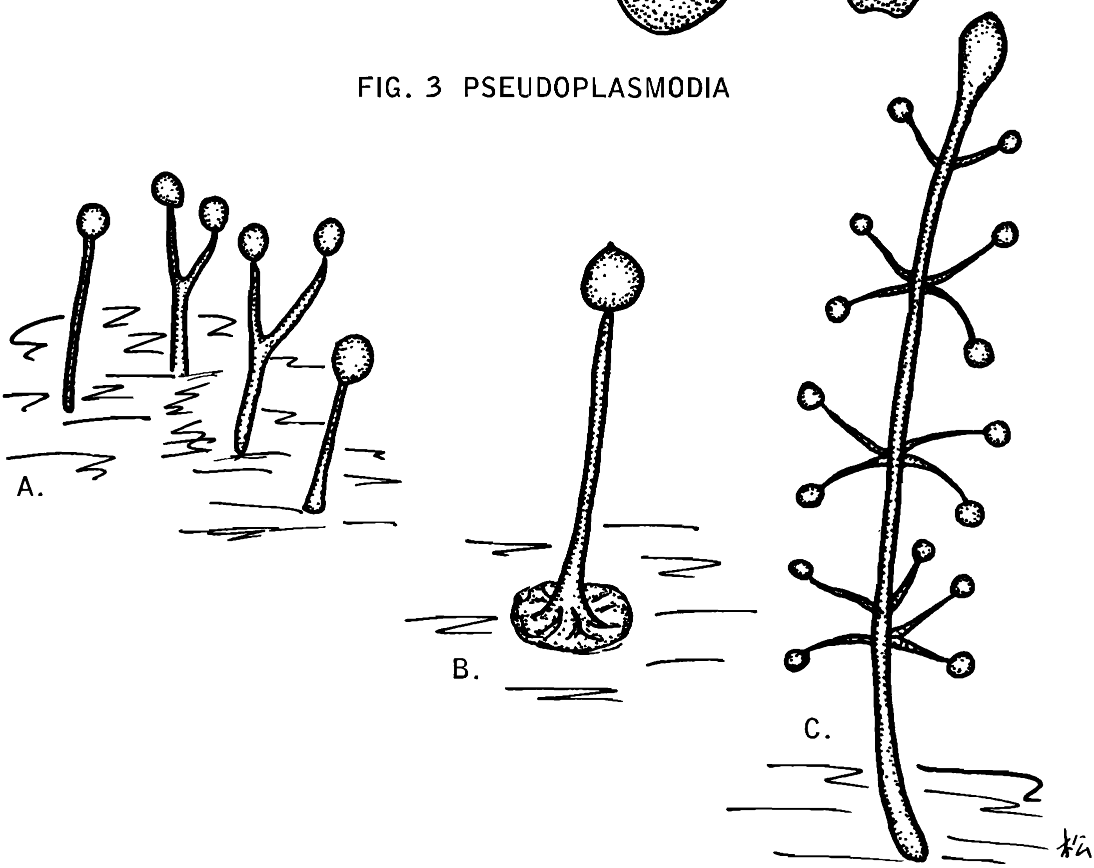


FIG. 4 THREE TYPES OF SOROCARPS

Plate I

Division (Phylum) Mycota - The Fungi

1. The fungi are nucleated, achlorophyllous organisms, which are generally filamentous, which generally reproduce both asexually and sexually, and whose somatic structures are typically surrounded by cell walls.

2. The fungi differ from the bacteria (Division Schizomycota) in size, being much larger, and, as a whole, in their more intricate structures, methods of reproduction, and life-cycles. Furthermore, the fungal nucleus is surrounded by a definite nuclear membrane and possesses one or more nucleoli, whereas the bacterial nucleus has neither a nuclear membrane nor a nucleolus.

3. The somatic phase of the fungi typically consists of a mass of branched filaments individually known as hyphae (sing. hypha) and collectively called the mycelium. There are, however, a number of fungal groups in which the soma is not mycelioid.

4. Fungi reproduce both asexually and sexually. Asexual methods of reproduction include simple cell division, budding, fragmentation, and the production of various types of spores. Spore production is particularly well developed in the fungi. Sexual reproduction, which includes plasmogamy, karyogamy, and meiosis, is initiated by various methods. The most common of these are: planogametic copulation, gametangial contact, spermatization, and somatogamy.

5. The division Mycota is subdivided into two sub-divisions: the Myxomycotina and the Eumycotina.

REFERENCES:

The following are general treatises on the fungi:

1. AINSWORTH, G. C. 1961. A Dictionary of the Fungi. viii + 547 pp. 138 figs. Commonwealth Mycological Institute, Kew, Surrey.
2. ALEXOPOULOS, C. J. 1962. Introductory Mycology. Ed. 2. John Wiley & Sons, Inc., New York.
3. BESSEY, E. A. 1950. Morphology and Taxonomy of Fungi. xiii + 791 pp. P. Blakiston Co. Philadelphia.
4. CATCHESIDE, D. G. 1951. Genetics of Microorganisms. vii + 223 pp., 35 figs. Sir Isaac Pitman and Sons, Ltd. London.
5. CHRISTENSEN, CLYDE M. 1961. The Molds and Man. Ed. 2, ix + 238 pp. University of Minnesota Press. Minneapolis.
6. CLEMENTS, F. E. and C. L. SHEAR. 1931. The Genera of Fungi. iv + 496 pp. H. W. Wilson Co. New York.
7. COCHRANE, V. W. 1958. Physiology of Fungi. xiii + 524 pp. John Wiley & Sons, Inc. New York.
8. CONANT, N. F., et al. 1954. Manual of Clinical Mycology. xii + 456 pp. 202 figs. Frontisp. W. B. Saunders Co., Philadelphia.
9. ENGLER, A. and K. PRANTL. Die natürlichen Pflanzenfamilien. Wilhelm Englemann, Leipsig. Three volumes deal with fungi as follows: 1897, Teil I, Abt. 1; 1907, Teil I, Abt. 1*; 1900, Teil I, Abt. 1**.
10. FOSTER, J. W. 1949. Chemical Activities of Fungi. xviii + 648 pp. Academic Press, Inc. New York.
11. GAUMANN, E. A. 1952. (English translation by F. L. Wynd.) The Fungi. Hafner, Publishing Co., New York.
12. GILMAN, J. C. 1957. Soil Fungi. x + 450 pp. 153 figs. Frontisp. Iowa State University Press, Ames.
13. GRAY, W. D. 1959. The Relation of Fungi to Human Affairs. xiii + 510 pp. 191 figs.
14. GWYNNE-VAUGHAN, HELEN and T. J. BARNES. 1948. Fungi. Ed. 2. xvi + 449 pp. Cambridge University Press. Cambridge.
15. HAWKER, LILLIAN. 1957. The Physiology of Reproduction in Fungi. viii + 128 pp. Cambridge University Press, Cambridge.
16. INGOLD, C. T. 1953. Dispersal in Fungi. vii + 197 pp. 90 figs. Clarendon Press, Oxford.
17. INGOLD, C. T. 1961. The Biology of Fungi. 124 pp. 61 figs. Hutchinson Educational Ltd., London.
18. KAMAT, M. N. 1959. Handbook of Mycology. Part I. ii + 185 pp. 13 figs. 15 pls. 1961. Part II. ii + 147 pp. 35 figs. 28 pls. Prakash Publishing House, Poona, India.
19. KELLEY, A. P. 1950. Mycotrophy in Plants. xvi + 223 pp. 16 figs. 5 pls. Frontisp. Chronica Botanica Co., Waltham, Mass.
20. LARGE, E. C. 1940. The Advance of the Fungi. 488 pp. 57 figs. 6 pls. Henry Holt and Co., New York.
21. LILLY, V. G. and H. L. BARNETT. 1951. Physiology of Fungi. xxii + 464 pp. McGraw-Hill Book Co., Inc. New York.
22. MARTIN, G. W. 1955. Are fungi plants? Mycologia, 47:779-792.
23. MOREAU, F. 1952-1953. Les Champignons. Encycl. Mycol. Vols. 22, 23. xv + 2120 pp. 1300 figs. Paul Lechevalier, Paris.

24. OLIVE, L. S. 1953. The structure and behavior of the fungus nuclei. Bot. Rev., 19:439-586.
25. RAMSBOTTOM, J. 1953. Mushrooms and Toadstools. xiv + 306 pp. 24 pls. 46 col. pls. Frontisp. Collins, London.
26. SACCARDO, P. A. 1882-1931. Sylloge Fungorum Omnium Hucusque Cognitorum. 25 vols. Published by the author, Pavia.
27. SKINNER, C. E., C. W. EMMONS, and H. M. TSUCHIYA. 1947. Molds, Yeasts, and Actinomycetes. xiv + 409 pp. John Wiley and Sons, Inc. New York.
28. STEVENS, F. L. 1925. Plant Disease Fungi. v + 469 pp. The Macmillan Co., New York.

GLOSSARY;

1. Budding (ME. budde = bud): the production of a small outgrowth (bud) from a parent cell. A method of asexual reproduction.
2. Fragmentation (L. frangere = to break): the segmentation of the thallus into a number of fragments each of which is capable of growing into a new individual.
3. Gametangial contact (Gr. gametes = husband + angeion = vessel): a method of sexual reproduction in which two gametangia come in contact but do not fuse. The male nucleus migrates through a pore or fertilization tube into the female gametangium.
4. Gametangial copulation (Gr. gametes = husband + angeion = vessel): a method of sexual reproduction in which the gametangia or their entire protoplasts fuse and give to rise to a zygote which develops into a resting spore or some other structure.
5. Gametangium (pl. gametangia; Gr. gametes = husband + angeion = vessel): a structure which contains gametes.
6. Karyogamy (Gr. karyon = nut, nucleus + gamos = marriage): the fusion of two compatible nuclei.
7. Hypha (pl. hyphae; Gr. hyphe = web): the unit of structure in the fungi; a tubular filament.
8. Meiosis (Gr. meiosis = reduction): a pair of nuclear divisions in quick succession one of which is reductional. Four haploid nuclei are produced as a result of meiosis.
9. Mycelium (pl. mycelia; Gr. mykes = mushroom): mass of hyphae constituting the body (thallus) of a fungus.
10. Planogamete (Gr. planetes = wanderer + gametes = husband): a motile gamete.
11. Planogametic copulation (Gr. planetes = wanderer + gametes = husband): fusion of naked gametes, one or both of which are motile.
12. Plasmogamy (Gr. plasma = a molded object + gamos = marriage): the fusion of two protoplasts.
13. Somatogamy (Gr. soma = body + gamos = marriage): fusion of somatic cells during plasmogamy.
14. Spermatium (pl. spermatia; Gr. spermation = little seed, sperm): a non-motile, uninucleate, spore-like male structure which empties its contents into a receptive female structure during plasmogamy.
15. Spermatization (Gr. sperma = seed): plasmogamy by the union of a spermatium with a receptive structure.

Sub-Division Myxomycotina – Class Myxomycetes (The True Slime Molds)

1. The true slime molds are organisms which exhibit characteristics intermediate between those of plants and those of animals. Their somatic phase is animal-like in that it possesses no cell walls; their propagative phase, on the other hand, is plant-like, consisting of spores each surrounded by a cell wall probably containing cellulose.

2. The somatic stage of the Myxomycetes is a plasmodium, a multinucleate mass of protoplasm, devoid of cell walls, with a creeping, flowing, amoeboid motion. The plasmodium feeds on bacteria, fungal spores, or fruiting bodies of fleshy fungi, such as mushrooms. It flows over the food material, engulfs it, and digests it in a truly animal fashion.

3. At the time of sporulation the entire plasmodium is converted into one or more fructifications which produce the spores. Meiosis typically takes place just before spore formation so that the spores are haploid.

4. At the time of germination a spore cracks open or develops a pore and the protoplast issues as a myxamoeba or a flagellated swarm cell. Swarm cells characteristically bear two anterior whiplash flagella.

5. Swarm cells and myxamoebae are interconvertible, the former withdrawing their flagella under dry conditions, the latter often developing flagella in a liquid medium.

6. Myxamoebae divide repeatedly producing large populations of haploid amoeboid cells. When a certain population density has been achieved, compatible myxamoebae or swarm cells fuse in pairs producing amoeboid or quadriflagellate zygotes respectively. Flagellated zygotes soon absorb their flagella becoming diploid myxamoebae. These are somewhat larger than the haploid myxamoebae but are difficult to distinguish from the latter.

7. Each zygote undergoes successive mitotic nuclear divisions and grows into a plasmodium in which all the nuclei are normally diploid. Plasmodia of the same species and strain fuse with each other and with zygotes upon contact. A plasmodium thus enlarges by growth and by incorporating other plasmodia.

8. The class Myxomycetes includes two sub-classes: the Ceratiomyxomycetidae, also called Exosporeae, and the Myxogastromycetidae, also called Myxogastres.

9. The following publications are useful in the identification of the Myxomycetes:

REFERENCES:

1. HAGELSTEIN, R. 1944. The Mycetozoa of North America. 306 pp. Illustr., Mineola, N. Y.
2. LISTER, A. and G. LISTER. 1925. A monograph of the Mycetozoa. xxxii + 296 pp. Illustr. Col. pls. Oxford Univ. Press. Oxford.
3. MARTIN, G. W. 1949. Fungi. Myxomycetes. North Am. Flora. Vol. 1, Pt. 1, 190 pp. N. Y. Bot. Garden. New York.
4. McBRIDE, T. H. and G. W. MARTIN. 1934. The Myxomycetes. viii + 339 pp. Illustr. Macmillan Company. New York.

EDUCATIONAL FILM:

Slime Molds III: Identification, U-5520, 24 min., sd color, 16 mm. film. (Technical direction by James L. Koevenig). Bureau of Audio-Visual Instruction, Extension Division, State University of Iowa, Iowa City, Iowa.

GLOSSARY:

1. Myxamoeba (pl. myxamoebae; Gr. myxa = slime + amoibe = change): an amoeboid cell, particularly one of the Myxomycetes.
2. Plasmodium (pl. plasmodia; Gr. plasma = a molded object): a naked, multinucleate mass of protoplasm moving and feeding in amoeboid fashion.
3. Swarm cell: A naked, amoeboid, flagellated cell.

CLASS MYXOMYCETES

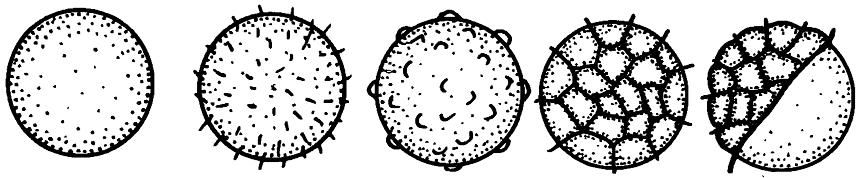


FIG. 5 VARIOUS TYPES OF SPORES

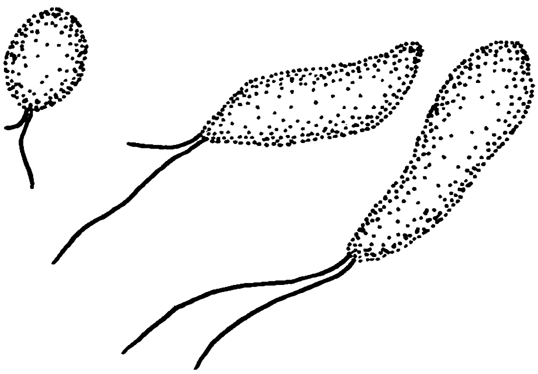


FIG. 6 SWARM CELLS

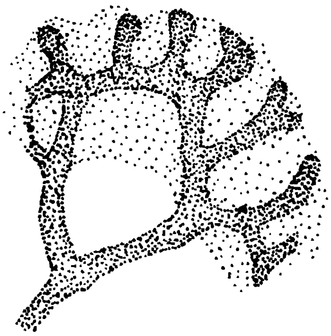


FIG. 7 PLASMODIUM

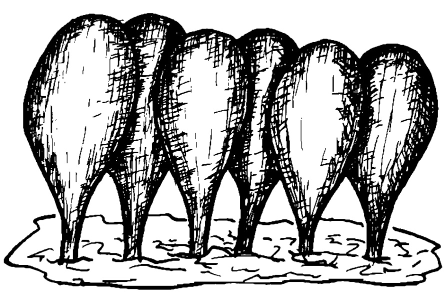


FIG. 8 SPORANGIA

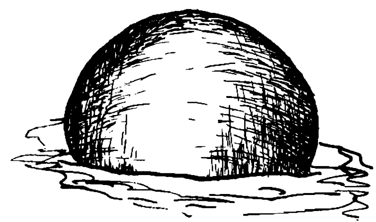


FIG. 9 AETHALIUM

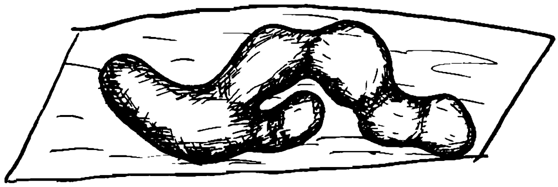


FIG. 10 PLASMODIOCARP

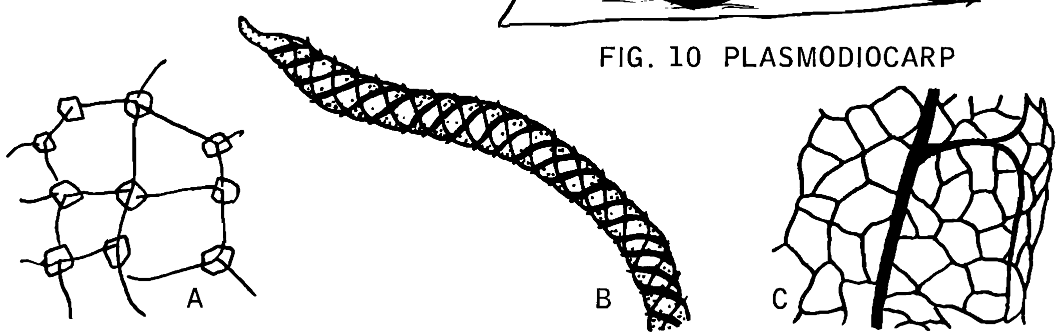


FIG. 11 THREE TYPES OF CAPILITIUM

Plate 2

Class Myxomycetes – Sub-Class Ceratiomyxomycetidae (Exosporeae)

A. GENERAL REMARKS:

1. The Ceratiomyxomycetidae produce their spores on the outside of a columnar fructification devoid of a peridium. This sub-class includes but a single order, the Ceratiomyxales, a single family, the Ceratiomyxaceae, and a single genus, *Ceratiomyxa*, with three species. One of these species, *Ceratiomyxa fruticulosa*, is universally distributed and of very common occurrence; the other two have been found only in the tropics.

B. PROCEDURE:

Order: Ceratiomyxales
Family: Ceratiomyxaceae
Genus: Ceratiomyxa

2. Examine the fructification of *Ceratiomyxa fruticulosa* with the unaided eye and under a dissecting microscope. Note the columnar, simple or branched structures which bear the spores externally. Learn to recognize this species at sight.

3. Mount a portion of one of the columns in lacto-phenol and study the attachment of the spores. Note the shape, color, and the wall of the spores.

4. If time permits make a hanging drop preparation of spores in distilled water and examine periodically for germination. Record your observations.

C. QUESTIONS:

1. Construct a life cycle diagram of *Ceratiomyxa fruticulosa*.
2. Where does meiosis take place in this organism according to Gilbert?
3. In accordance with these findings, to what structure of the Myxogastromycetidae is the "spore" of the Ceratiomyxales homologous?
4. Is it etymologically correct, accordingly, to call this sub-class Exosporeae?

D. TEXT REFERENCES:

Alexopoulos -- pp. 67-71.
Bessey -- pp. 26-27.

E. SELECTED REFERENCES:

1. ALEXOPOULOS, C. J. 1963. The Myxomycetes II. Botanical Review.
2. GILBERT, H. C. 1935. Critical events in the life history of *Ceratiomyxa*. Am. Jr. Bot. 22:52-74.
3. MARTIN, G. W. 1940. The Myxomycetes. Bot. Rev. 6:356-388.
4. MC MANUS, SISTER M. A. 1958. In vivo studies of plasmogamy in *Ceratiomyxa*. Bull. Torrey Bot. Club., 85:28-37.

Class Myxomycetes–
Sub-Class Myxogastromycetidae

A. GENERAL REMARKS:

1. The Myxogastromycetidae (also called Endosporeae and Myxogastres) produce their spores inside a fruiting structure which is covered, at least in the early stages, by a peridium. Three types of fructifications are recognized: the sporangium, the aethalium, and the plasmodiocarp.

2. In accordance with the newer classification, this sub-class is sub-divided into five orders on the basis of spore color, presence or absence of capillitium, and presence or absence of lime in various parts of the fructification.

Order: Physarales

A. GENERAL REMARKS:

3. In the Order Physarales, the spores are black or deep violet in mass. Lime is characteristically present in the capillitium or in the peridium, or in both. Two families are now recognized: the Physaraceae and the Didymiaceae.

Family: Physaraceae

Genus: Physarum

B. PROCEDURE:

4. Observe a living, young phaneroplasmodium of *Physarum polycephalum* or other member of this family growing on a thin layer of agar in a Petri dish. Note the color, the consistency, and the general structure. Invert the Petri dish on the microscope stage and observe the streaming of the plasmodial protoplast under low power objective. Note the "veins" in which the streaming occurs. Watch the protoplasm for some seconds and note the sudden reversal of the direction of streaming which will take place. Time the protoplasmic flow in each direction several times and calculate the average number of seconds between reversals. Now turn off the microscope lamp for a given number of seconds; turn on the light again and observe any change in the direction of flow. Repeat several times and note if you can increase the frequency of reversal of flow through the light stimulus. Using a wax pencil, outline the edge of the plasmodium on the bottom of the Petri dish, and observe periodically to determine change of position of the plasmodial mass.

5. Cultivate this plasmodium according to Camp's method and study its development and eventually its sporulation.

6. Study the fructification of *Physarum polycephalum* or of any other species of *Physarum* with a sporangial fructification. Note the hypothallus, if present, the stalk, and the sporangium. Note the large quantities of lime generally present in the peridium. Lime in the capillitium is in the form of nodules connected by hyaline threads. Presence of lime in the capillitium is characteristic of the family Physaraceae.

7. Mount a portion of the capillitium and spore mass in distilled water or dilute detergent. Observe the lime nodules and note their shape and size. These are characters used in distinguishing between species of this large genus.

Genus: Badhamia

8. Study the fructification of a species of Badhamia under the dissecting microscope and note the appearance of the capillitium as distinguished from that of Physarum.

9. Using any one of the standard keys for the determination of Myxogastres, identify as many species of the Order Physarales as time and material available permit.

Genus: Fuligo

10. Observe the fructification of Fuligo septica. This is an aethalium. Note the abundance of lime. Make a water mount from the interior of the fructification and study the spores and the capillitium.

11. Make a hanging drop preparation of some resting spores of Fuligo septica which have been previously treated with one of the bile salts. Observe periodically during the laboratory period for germination. Watch the cracking of the coat of a germinating spore and the emergence of the swarm cell or cells. If you regulate your light and observe carefully you should be able to see the two flagella at the anterior end of the swarm cell. Note the shape of the swarm cells and their type of movement. Store your mount until the next laboratory period in a moist chamber and then search the hanging drop for copulating cells and zygotes.

Family: Didymiaceae

Genus: Didymium

12. Study the sporangia of Didymium iridis or other species of this genus. Observe how the capillitium differs from that of the Physaraceae. Examine some peridial lime under the microscope. Note that it is crystalline. This character is of considerable taxonomic importance (Martin, 1949).

13. Spread some spores of Didymium iridis on half-strength corn meal agar (equal quantities of Difco corn meal agar without dextrose, and 2% plain agar). Pour 5 ml. sterile distilled water over the spores and incubate for four or five days. Examine the cultures for the presence of plasmodia. These may be minute and detectable only under the dissecting microscope.

14. When plasmodia have developed, feed the culture by sprinkling some pulverized sterile oat flakes over the agar. When vigorous plasmodia have been obtained, transfer an agar block bearing a well-developed plasmodial fan to plain agar and examine from time to time for the development of sporangia.

C. QUESTIONS:

1. Characterize the Myxomycetes.
2. How do the true slime molds differ from the cellular slime molds?
3. Discuss the feeding habits of the Myxomycetes. Would you call the Myxomycetes saprobic, parasitic, holozoic, or holophytic?

4. Construct a life cycle diagram of Physarum polycephalum.
5. If you plant a single spore of one of the Myxomycetes on a favorable medium would you expect the development of plasmodia? Discuss. (See Dee, 1960; Collins, 1961.)
6. Characterize the Physaraceae; the Didymiaceae.
7. Why is water used as a mounting medium instead of lactophenol when studying the Physarales?
8. How good a taxonomic criterion is color of plasmodium?
9. Characterize the plasmodium of the Physarales. (See Alexopoulos, 1960/1961; McManus and Taylor, 1961/1962.)

D. TEXT REFERENCES:

Alexopoulos -- pp. 71-92; 95.
Bessey -- pp. 22-26; 27-28.

E. SELECTED REFERENCES:

1. ALEXOPOULOS, C. J. 1960 (1961). Gross morphology of the plasmodium and its possible significance in the relationships among the Myxomycetes. Mycologia, 52:1-20.
2. ALEXOPOULOS, C. J. 1963. The Myxomycetes. II. Bot. Rev. (In Press).
3. ALEXOPOULOS, C. J. and G. G. ZABKA. 1962. Production of hybrids between physiological races of the true slime mould Didymium iridis. Nature (London), 193:598-599.
4. CAMP, W. G. 1936. A method of cultivating myxomycete plasmodia. Bull. Torrey Bot. Club. 63:205-210.
5. CAMP, W. G. 1937. The fruiting of Physarum polycephalum in relation to nutrition. Am. Jr. Bot., 24:300-303.
6. COHEN, A. L. 1939. Nutrition of the Myxomycetes. I. Pure culture and two-membered culture of myxomycete plasmodia. Bot. Gaz., 101:243-275.
7. COLLINS, O. R. 1961. Heterothallism and homothallism in two Myxomycetes. Am. Jr. Bot., 48:674-683.
8. CROWDER, W. 1926. Marvels of Mycetozoa. Nat. Geogr. Mag., 49:421-443.
9. DANIEL, J. W. and H. P. RUSCH. 1961. The pure culture of Physarum polycephalum on a partially defined soluble medium. Jr. Gen Microbiol., 25:47-59.
10. DANIEL, J. W. and H. P. RUSCH. 1962. Method for inducing sporulation of pure cultures of the myxomycete Physarum polycephalum. Jr. Bact., 83:234-240.
11. DEE, JENIFER. 1960. A mating type system in an acellular slime mould. Nature (London), 185:780-781.
12. ELLIOTT, E. W. 1949. The swarm cells of Myxomycetes. Mycologia, 41:141-170.
13. GILBERT, F. A. 1928. Feeding habits of the swarm cells of the myxomycete Dictydiaethalium plumbeum. Am. Jr. Bot., 15:123-131.
14. GILBERT, F. A. 1928. Observations on the feeding habits of the swarm cells of Myxomycetes. Am. Jr. Bot., 15:345-352.
15. GRAY, W. D. 1938. The effect of light on the fruiting of Myxomycetes. Am. Jr. Bot., 25:511-522.
16. GRAY, W. D. 1939. The relation of pH and temperature to the fruiting of Physarum polycephalum. Am. Jr. Bot., 26:709-714.
17. GRAY, W. D. 1953. Further studies on the fruiting of Physarum polycephalum. Mycologia, 45:817-824.
18. GUTTES, E., S. GUTTES, and H. P. RUSCH. 1961. The morphology and development of Physarum polycephalum grown in pure culture. Devel. Biol., 3:588-614.
19. HARPER, R. A. and B. O. DODGE. 1914. The formation of capillitium in certain Myxomycetes. Ann. Bot., 28:1-18.
20. HOWARD, F. L. 1931. The life history of Physarum polycephalum. Am. Jr. Bot., 18:116-133.
21. JUMP, J. A. 1954. Studies on sclerotization in Physarum polycephalum. Am. Jr. Bot., 41:561-567.
22. KAMBLY, P. E. 1939. The color of myxomycete plasmodia. Am. Jr. Bot., 26:386-390.
23. KAMIYA, N. 1959. Protoplasmic streaming. Protoplasmatologia, vol. 8, pt. 3a, 199 pp.
24. KERR, N. S. 1960. Flagella formation by myxamoebae of the true slime mold Didymium nigripes. Jr. Protozool., 7:103-108.
25. MC MANUS, SISTER M. A. and MARY K. TAYLOR. 1961 (1962). Some observations on the plasmodia of Myxomycetes. Proc. Iowa Acad. Sci., 68:72-78.
26. MARTIN, G. W. 1940. The Myxomycetes. Bot. Rev., 6:356-388.
27. MARTIN, G. W. 1960 (1961). The systematic position of the Myxomycetes. Mycologia, 52:119-129.
28. NYGAARD, O. F., SOPHIA GUTTES, and H. P. RUSCH. 1959. Nucleic acid metabolism in a slime mold with synchronous mitosis. Biochim et Biophys. Acta, 38:298-306.
29. RAKOCZY, I. 1961. Observations on the regeneration of the plasmodium of the myxomycete Didymium xanthopus (Ditm.) Fr. Acta Soc. Bot. Polon., 30:443-456.
30. ROSS, I. K. 1957. Syngamy and plasmodium formation in the Myxomycetes. Am. Jr. Bot., 44:843-850.
31. ROSS, I. K. 1961. Further studies on meiosis in the Myxomycetes. Am. Jr. Bot., 48:244-248.
32. WELDEN, A. L. 1955. Capillitial development in the Myxomycetes Badhamia gracilis and Didymium iridis. Mycologia, 47:714-728.
33. WILSON, C. L. and I. K. ROSS. 1957. Meiosis in the Myxomycetes. Am. Jr. Bot., 42:743-749.

F. EDUCATIONAL FILMS:

Slime Molds I: Life cycle, U-5518, 30 min. sd. color, 16 mm. film. (Technical direction by James L. Koevenig). Bureau of Audio-Visual Instruction. Extension Division, State University of Iowa, Iowa City, Iowa.

Slime Molds II: Collection, cultivation, and use, U-5519, 19 min., sd. color, 16 mm. film. (Technical direction by James L. Koevenig). Bureau of Audio-Visual Instruction, Extension Division, State University of Iowa, Iowa City, Iowa.

Slime Molds III: Identification, U-5520, 24 min., sd. color 16 mm. film. (Technical direction by James L. Koevenig). Bureau of Audio-Visual Instruction, Extension Division, State University of Iowa, Iowa City, Iowa.

G. GLOSSARY:

1. Aethalium (pl. aethalia; Gr. aithalos = soot): a rather large, sometimes massive, generally cushion-shaped fructification of some Myxogastres.
2. Capillitium (pl. capillitia; L. capillus = hair): sterile, thread-like structures present among the spores in the fruiting bodies of many Myxogastres.
3. Hypothallus (pl. hypothalli; Gr. hypo = under + thallos = shoot, thallus): a thin, often transparent deposit at the base of the fructifications of some Myxogastres.
4. Plasmodiocarp (Gr. plasma = something molded + karpos = fruit): a curved or branched, vein-like fruiting structure of some Myxogastres.
5. Sporangium (pl. sporangia; Gr. sporos = seed, spore + angeion = vessel): a structure, generally a single cell, which contains one or more spores. Generally an asexual structure.

Class Myxomycetes– Sub-Class Myxogastromycetidae

Order: Stemonitales

A. GENERAL REMARKS:

1. The spores of the Stemonitales are typically brown or deep violet in mass. Lime is conspicuously absent except in a few forms in which it is restricted to the hypothallus, stipe, or columella. Two families are recognized: the Stemonitaceae and the Collodermataceae. The first of these is the largest and the most frequently collected.

B. PROCEDURE:

Family: Stemonitaceae

Genus: Stemonitis

2. Observe a colony of Stemonitis growing on wood. Note the numerous sporangia arising from a common hypothallus. Note that no peridium can be seen in most sporangia.

3. By means of a sharp pair of forceps, separate a single sporangium from the colony and mount in lacto-phenol. Study the stalk, columella, capillitium, and spores. Use the oil immersion objective to determine the markings, if any, on the spore wall.

4. Make a hanging drop preparation of Stemonitis spores in water. Observe from time to time for germination.

5. Spread spores of Stemonitis fusca, taken from sporangia developed during the current or previous season, on the surface of half-strength corn meal agar and pour 10 ml. sterile distilled water over them. Examine the culture under the low power objective of your compound microscope after four or five days and search for small plasmodia. Note their structure, their characteristic general shape, and their transparency. This type of plasmodium is called the aphanoplasmodium. Follow the growth and development of the plasmodia over a period of several days. Add water from time to time as needed to keep the plasmodia submerged. If the culture is permitted to dry in its early stages of development, the plasmodia will sclerotize.

6. After the plasmodia have developed well, gradually reduce the amount of water until finally the surface of the agar is dry. Expose the culture to bright light, daylight or artificial, but avoid excessive heat from either direct sunlight or hot electric light bulb.

7. If the cultures fruit, follow the development of the fruiting bodies.

Genera: Comatricha, Diachea, Lamproderma

8. Study other species of Stemonitis as time permits, as well as representatives of Comatricha, Diachea, and Lamproderma. Note the presence of lime in the hypothallus and stalk of Diachea.

9. Using one of the standard keys, identify as many species as time permits.

C. QUESTIONS:

1. How do the Stemonitales differ from the Physarales?
2. Why is the peridium of Stemonitis difficult to find? Is this true of the entire order?
3. What is the relationship of the capillitium of the Myxogastres to the spores? To the columella?
4. What is the relationship of the columella to the stalk?
5. Describe an aphanoplasmodium. How does it differ from the plasmodium of the Physarales?
6. By what method do the spores of Stemonitis fusca germinate?
7. How does spore germination in Stemonitis differ from that in Fuligo?

D. TEXT REFERENCES:

Alexopoulos -- pp. 94-95.
Bessey -- pp. 22-28.

E. SELECTED REFERENCES:

1. ALEXOPOULOS, C. J. 1959. The laboratory cultivation of Stemonitis. Am. Jr. Bot., 46:140-142.
2. ALEXOPOULOS, C. J. 1960 (1961). Gross morphology of the plasmodium and its possible significance in the relationships among the Myxomycetes. Mycologia, 52:1-20.
3. DENNISON, MARY LOUISE. 1945. The genus Lamproderma and its relationships. I. Mycologia, 37:80-108; II. Mycologia, 37:197-204.
4. GOODWIN, DONNA. 1961. Morphogenesis of the sporangium of Comatrica. Am. Jr. Bot., 48:148-154.
5. MC MANUS, SISTER M. A. 1961. Culture of Stemonitis fusca on glass. Am. Jr. Bot., 48:582-588.
6. MC MANUS, SISTER M. A. 1961. Laboratory cultivation of Clastoderma debaryanum. Am. Jr. Bot., 48:884-888.
7. MC MANUS, SISTER M. A. and SISTER M. V. RICHMOND. 1961. Spore to spore culture on agar of Stemonitis fusca. Am. Midl. Nat., 65:246.
8. ROSS, I. K. 1957. Capillitial formation in the Stemonitaceae. Mycologia, 49:809-819.
9. ROSS, I. K. 1961. Sporangial development in Lamproderma arcyrionema. Mycologia, 49:809-819.

F. GLOSSARY:

1. Aphanoplasmodium (pl. aphanoplasmodia; Gr. aphanes = invisible, inconspicuous + plasmodium): a plasmodium consisting, in its early stages, of a network of very fine, transparent strands not conspicuously differentiated into ecto- and endoplasm, and in which the protoplasm is not coursely granular. Characteristic of Stemonitis and related genera.
2. Columella (pl. columellae; L. columen = column): a sterile structure within a sporangium or other fructification; often an extension of the stalk.

Class Myxomycetes – Sub-Class Myxogastromycetidae

Order: Echinosteliales

A. GENERAL REMARKS:

1. This very small order, consisting of the single family Echinosteliaceae which includes the single genus Echinostelium, was first established by Martin in 1960. The family Echinosteliaceae, up to that time had been included in the Stemonitales.

2. The Echinosteliales belong to the group of Myxomycetes with colorless or brightly colored spores. The plasmodium, in the three species in which it is known, is a protoplasmodium which gives rise to but a single fructification. The capillitium varies from completely absent to a well-developed, open, globose net. A columella is almost always present and is sometimes very conspicuous; it may, however, be absent.

Family: Echinosteliaceae

Genus: Echinostelium

B. PROCEDURE:

3. Collect several small pieces (about 1 x 2 inches) of bark from a living elm tree and place them on discs of filter paper in Petri dishes. Pour distilled water over the bark and allow to soak overnight. Pour off the excess water, replace the Petri dish cover, and permit the bark to remain in the moist chamber for several days. Examine the bark periodically through the Petri dish cover under the dissecting microscope for the presence of minute sporangia of Echinostelium minutum one of four known species in this genus.

4. Mount an entire sporangium in lacto-phenol or in lacto-phenol-cotton-blue and study the capillitium and the spores under the oil immersion objective. Observe the thickened areas on the spore walls.

5. Attempt cultures of the organism on corn meal agar together with Escherichia coli or Aerobacter aerogenes. If plasmodia are produced study their morphology and the streaming of the protoplasm. Observe the fruiting process.

C. QUESTIONS:

1. How does a protoplasmodium differ from the other two types of plasmodia you have already studied?
2. In what species of Myxomycetes have protoplasmodia been reported?
3. Does the sporangium of Echinostelium have a peridium?
4. Name some species of Myxomycetes which often develop on bark in moist chamber culture. Why are such species seldom found in the field?

D. TEXT REFERENCES:

Alexopoulos -- pp. 93-94.

E. SELECTED REFERENCES:

1. ALEXOPOULOS, C. J. 1958. Three new species of Myxomycetes from Greece. Mycologia, 50:52-56.
2. ALEXOPOULOS, C. J. 1960a. Morphology and laboratory cultivation of Echinostelium minutum deBary. Am. Jr. Bot., 47:37-43.
3. ALEXOPOULOS, C. J. 1960b (1961). Gross morphology of the plasmodium and its possible significance in the relationships among the Myxomycetes. Mycologia, 52:1-20.
4. ALEXOPOULOS, C. J. 1961. A new species of Echinostelium from Greece. Am. Midl. Nat., 66:391-394.
5. LISTER, A. and GULIELMA LISTER 1925. A monograph of the Mycetozoa. Ed. 3. xxxii + 296 pp. Illustr. Col. pls. Brit. Museum (Nat. Hist.), London.
6. MARTIN, G. W. 1960. (1961). The systematic position of the Myxomycetes. Mycologia, 52:119-129.
7. NANNENGA-BREMEKAMP, N. E. 1961. Notes on Myxomycetes IV. Acta Bot. Neerland., 10:55-66.

F. GLOSSARY:

1. Protoplasmodium (pl. protoplasmodia; Gr. protos = first + plasmodium): a microscopic plasmodium, with no differentiated fan-shaped region or strands, which exhibits slow and irregular streaming and which gives rise to but a single, minute fructification. Typical of the Echinosteliales but occurring in other Myxomycetes also.

Class Myxomycetes– Sub-Class Myxogastromycetidae

Order: Liceales

A. GENERAL REMARKS:

1. The Liceales possess pallid or bright colored spores (rarely dark). True capillitium is lacking, but pseudocapillitium, in the form of tubules, perforated plates, etc., may be present. Considerable experience is required to differentiate between capillitium and pseudocapillitium in some species.

2. Many of the Liceales which produce minute fruiting bodies, will fruit on bark of living trees placed in a moist chamber in accordance with the method described under the Genus Echinostelium in the previous exercise.

3. The order is subdivided into three families by Martin (1949): the Liceaceae, the Reticulariaceae, and the Cribrariaceae.

B. PROCEDURE:

4. Study the fructifications of two or more representative genera in the Liceales. Dictydium in the Cribrariaceae and Lycogala in the Reticulariceae are perhaps the most common.

Family: Cribrariaceae

Genus: Dictydium

5. Mount a fructification of Dictydium cancellatum in lacto-phenol. This is another example of the sporangial type of fructification. Note the distinct longitudinal ribs connected with finer horizontal threads. Note the color and structure of the spores.

Family: Reticulariaceae

Genus: Lycogala

6. Study the fructification of Lycogala epidendrum. This is another example of the aethalial type of fructification. Puncture the peridium and mount a portion of the spore mass in lacto-phenol. Note the pseudocapillitium. Study the structure of the spore wall. Learn to recongnize the conspicuous coral-colored plasmodium of this species.

C. QUESTIONS:

1. What are the "ribs" in the sporangium of Dictydium?
2. What is an aethalium? How is it formed?
3. How does pseudocapillitium differ from capillitium?

D. TEXT REFERENCES:

Alexopoulos -- pp. 92.
Bessey -- pp. 22-28.

E. SELECTED REFERENCES:

1. BAKER, GLADYS E. 1932. A comparative morphological study of the Myxomycete fructification. Univ. Iowa Stud. Nat. Hist., 16:1-56.
2. CONARD, H. S. 1910. Spore formation in *Lycogala exiguum* Morg. Proc. Iowa Acad. Sci., 17:83-84.
3. WILSON, W. and E. J. CADMAN. 1928. The life history and cytology of *Reticularia Lycoperdon*. Trans. Royal Soc. Edinb., 55:555-608.

F. GLOSSARY:

1. *Pseudocapillitium* (pl. *pseudocapillitia*; Gr. *pseudo* = false + capillitium): irregular threads, plates or other structures present among the spores within the fructifications of many Myxogastres; resembles capillitium.

Class Myxomycetes – Sub-Class Myxogastromycetidae

Order: Trichiales

A. GENERAL REMARKS:

1. The spores of the Trichiales are pallid or bright colored. The capillitium is generally abundant and consists of sculptured, free or attached threads. These may be short, in the form of free elaters or may form a network.

2. The order is divided into two families: the Dianemaceae and the Trichiaceae. The latter is by far the largest and most common.

B. PROCEDURE:

Family: Trichiaceae

Genera: Arcyria, Trichia, Hemitrichia

3. Study the fructification of at least one species of each of the three most common genera of the Trichiaceae: Arcyria, Trichia, and Hemitrichia. If Hemitrichia serpula is available compare its fruiting body -- a plasmodiocarp -- with others you have studied.

4. Mount a portion of the capillitium of each of the three genera listed above in lacto-phenol and make a comparative study. The capillitial characteristics are used to distinguish between these three genera.

5. Identify as many species in the Trichiales as time and material permit.

C. QUESTIONS:

1. Distinguish between Trichia, Hemitrichia, and Arcyria.
2. Construct a key to the four order of the Myxogastres.
3. Distinguish between the three types of fructifications produced by the Myxogastres
4. Discuss Martin's views on the systematic position of the Myxogastres and compare and contrast them with those of other authors.

D. TEXT REFERENCES:

Alexopoulos -- pp. 92-93.
Bessey -- pp. 22-28

E. SELECTED REFERENCES:

1. NAUSS, RUTH N. 1943. Observations on the culture of Hemitrichia vesparium, with special reference to its black plasmodial color. Bull. Torrey Bot. Club, 70:152-163.
2. NAUSS, RUTH N. 1947. My garden of slime molds. Jr. N. Y. Bot. Gard., 48:101-109.

F. GLOSSARY:

1. Elaters (Gr. elater = driver): short, rope-like structures interspersed with the spores of some Myxomycetes, constituting the capillitium. Possible aid in spore dispersal.

Sub-Division Eumycotina – The True Fungi

1. In contrast to the Sub-division Myxomycotina, the Eumycotina, with few exceptions, possess a cellular thallus. This may be unicellular or multicellular and is characteristically walled.

2. The Sub-division Eumycotina contains 8 classes and 2 form-classes of fungi. Whereas classes are considered to be natural classification units, form-classes are admittedly artificial and are maintained only as a matter of convenience.

3. Six of the 8 classes in the Eumycotina comprise what are often called the Lower Fungi or Phycomycetes. They are: the Chytridiomycetes, Hyphochytridiomycetes, Oömycetes, Plasmodiophoromycetes, Zygomycetes, and Trichomycetes. The Higher Fungi consist of the classes Ascomycetes and Basidiomycetes and of the form-class Deuteromycetes. Another group of organisms, composed of fungi and algae in association, is known as the form-class Lichenes.

4. As mentioned above, the first six classes of the sub-division Eumycotina are often grouped under the name Phycomycetes. Fungi in these classes typically possess a coenocytic mycelium devoid of cross walls, the protoplasm being continuous and multinucleate. Asexual reproduction is usually by the formation of sporangia which enclose indefinite numbers of spores, but conidia (asexual spores produced on the hyphae) are formed by some species. Sexual reproduction characteristically results in the formation of resting spores.

5. The classes of fungi included in the Eumycotina are characterized as follows:

   a. Chytridiomycetes, with posteriorly whiplash, uniflagellate zoöspores, varied types of thalli, and varied types of sexual reproduction.

   b. Hyphochytridiomycetes have anteriorly uniflagellate zoöspores with tinsel flagella, varied types of thalli, and only one known incidence of sexual reproduction.

   c. Oömycetes develop biflagellate zoöspores with oppositely directed, nearly equal, flagella (the forwardly directed of the tinsel type, the posteriorly directed of the whiplash type), well-developed mycelium in most instances, and oögamous sexual reproduction.

   d. Plasmodiophoromycetes have unequally biflagellate zoöspores, with both flagella of the whiplash type, and a plasmodial thallus.

   e. Zygomycetes are characterized by a well-developed mycelium, a terrestrial habitat, nonmotile spores, and thick-walled zygospores formed by fusion of two, usually equal, gametangia.

   f. Trichomycetes are attached to the intestinal tract or the exoskeleton of arthropods. Their mycelium is not as well-developed as that of the Zygomycetes. Asexual reproduction is by means of sporangiospores or conidia.

   g. Ascomycetes have a septate mycelium consisting of uninucleate or multinucleate cells. The septa are perforated in the center and permit strands of protoplasm to pass from cell to cell. The typical reproductive structure of the Ascomycetes

is the ascus (pl. asci), a sac-like structure which generally contains 8 ascospores formed by sexual reproduction.

h. Basidiomycetes have a septate mycelium consisting of uninucleate or binucleate cells. The septa are perforated in the center. The binucleate mycelium, which arises through sexual reproduction, characteristically bears clamp connections. The typical reproductive structure of the Basidiomycetes is the basidium (pl. basidia), which bears four basidiospores on its surface. Basidiospores are formed through sexual reproduction, and correspond to the ascospores of the Ascomycetes.

i. Deuteromycetes are an artificial group (form-class) in which we place fungi which, so far as is known, do not possess a true sexual cycle. Asexual stages of Ascomycetes and some Basidiomycetes are also placed here for convenience in identification.

j. Lichenes, commonly called lichens, is a form-class consisting of a large group of dual organisms in which fungi and algae, in close association, form characteristic thalli.

GLOSSARY:

1. Ascospore (Gr. askos = sac + sporos = seed, spore): a spore which results from meiosis, borne in an ascus.
2. Ascus (pl. asci; Gr. askos = sac): a sac-like structure containing a definite number of ascospores, usually 8, which are typically formed as a result of karyogamy and meiosis taking place in the young ascus.
3. Basidiospore (Gr. basidion = a small base + spores = seed, spore): a spore which results from meiosis, borne on a basidium.
4. Basidium (pl. basidia; Gr. basidion = a small base): a structure bearing on its surface a definite number of basidiospores -- typically 4 -- which are usually formed as a result of karyogamy and meiosis in the young basidium.
5. Clamp connection: a bridge-like, hyphal connection characteristic of the secondary (binucleate) mycelium of the Basidiomycetes.
6. Coenocytic (Gr. koinos = common + kytos = a hollow vessel): not septate; referring to the fact that the nuclei are embedded in the cytoplasm without being separated by cross walls, i. e. , the nuclei lie in a common matrix.
7. Conidium (pl. conidia; Gr. konis = dust + -idion = dimin. suffix): a spore formed asexually, usually at the tip, side, or inside (endoconidium) a hypha.
8. Flagellum (pl. flagella; L. flagellum = whip): a hair-, whip-, or tinsel-like structure which serves to propel a motile cell.
9. Gametangium (pl. gametangia; Gr. gametes = husband + angeion = vessel): a structure which contains gametes.
10. Septate (L. septum = hedge, partition): separated by cross walls.
11. Septum (pl. septa; L. septum = hedge, partition): a cross wall in a hypha.
12. Zoöspore (Gr. zoön = animal + sporos = seed, spore): a motile spore equipped with cilia or flagella.
13. Zygospore (Gr. zygos = yoke + sporos = seed, spore): a resting spore which results from the union of two gametangia.
14. Zygote (Gr. zygos = yoke): a diploid cell resulting from the union of two haploid cells. A cell in which karyogamy has taken place.

Class Chytridiomycetes

A. GENERAL REMARKS:

1. In the Chytridiomycetes, all of the orders have zoöspores with a posteriorly located whiplash flagellum. The thallus varies in this group. Some species may be plasmodial for a period of time; others may be one-celled or have rudimentary hyphae with rhizoids; still others may be mycelial in form. The mycelium, when present, is non-septate, or coenocytic, and the protoplasm is continuous and multinucleate. In asexual reproduction the zoöspores are formed in a sporangium. Sexual reproduction results in the formation of a resting sporangium or a resting spore.

2. This class includes three orders: the Chytridiales, the Blastocladiales and the Monoblepharidales.

Order Chytridiales

A. GENERAL REMARKS

3. The Chytridiales are considered the most primitive of the Phycomycetes. They may be unicellular and holocarpic or they may be eucarpic possessing a rhizoidal system and one or more reproductive organs; none has true mycelium.

4. A large number of the Chytridiales are parasitic in fresh-water algae, in aquatic fungi, pollen grains, decaying plant tissues, small aquatic animals, and a few are parasitic in terrestrial or aquatic vascular plants. A few have been reported in marine algae.

5. This order is divided into 8 families by Sparrow (1960). Six of these are discussed below.

B. PROCEDURE:

Family: Olpidiaceae

Genus: Olpidium

6. Examine some fresh-water algae, pollen grains, eggs or other submerged materials infected with Olpidium sp. on a slide mount. Find the spherical or ellipsoidal, inoperculate sporangia inside the host. Locate the discharge tube and pore for discharge of zoöspores. Find the thick-walled resting spore that usually contains a large globule.

Genus: Rozella

7. Examine hyphae or Achlya sp. which have been parasitized by Rozella achlyae. (Achlya is a water mold in the order Saprolegniales.) Note the hypertrophy of the parasitized hyphal tips.

CLASS CHYTRIDIOMYCETES

SOMATIC STRUCTURES

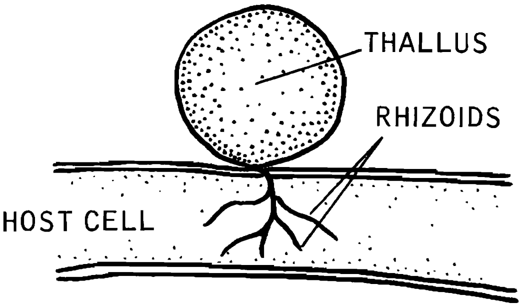


FIG. 12 THALLUS WITH RHIZOIDS

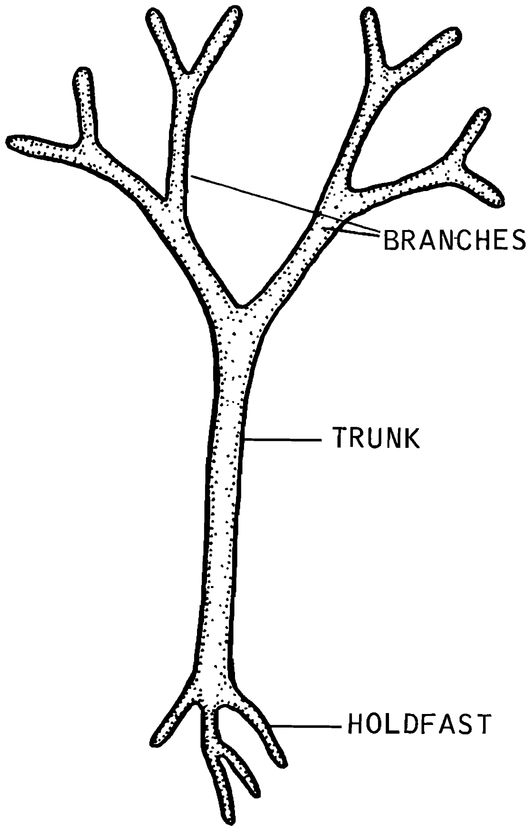


FIG. 14 LIMITED MYCELIAL THALLUS

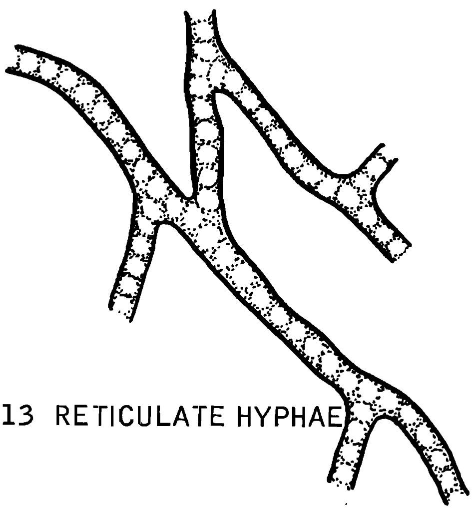


FIG. 13 RETICULATE HYPHAE

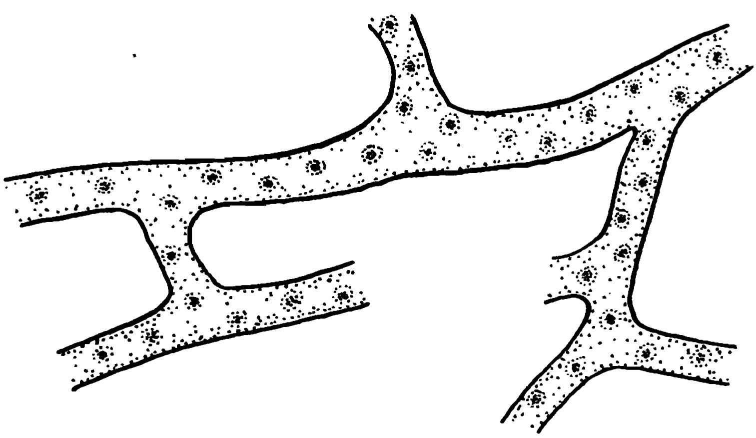


FIG. 15 TYPICAL HYPHA OF HIGHER PHYCOMYCETES

Plate 3

CLASSES CHYTRIDIOMYCETES (FIG. 16)
OÖMYCETES (FIGS. 17-19) ZYGOMYCETES (FIGS. 20-22)

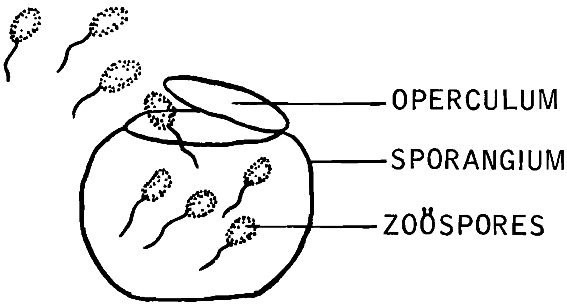


FIG. 16 OPERCULATE SPORANGIUM

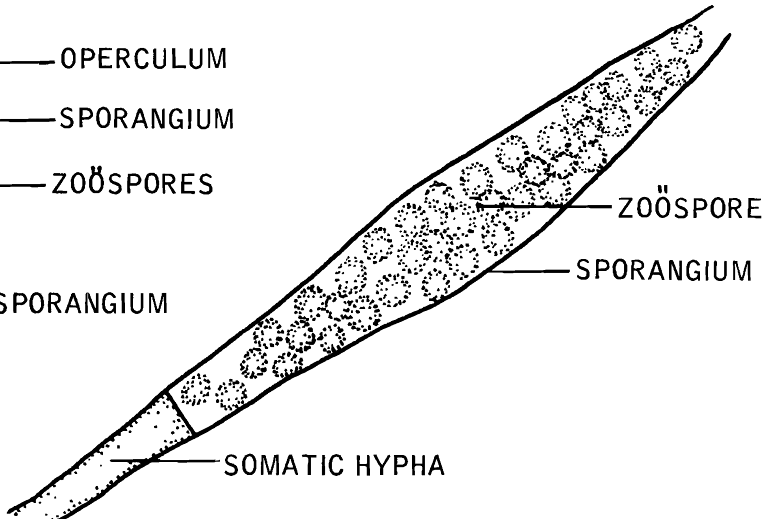


FIG. 17 ELONGATED SPORANGIUM

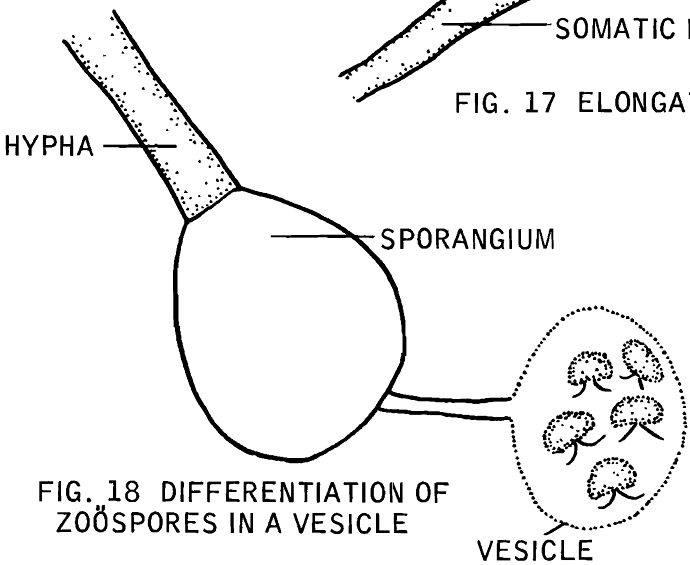


FIG. 18 DIFFERENTIATION OF ZOÖSPORES IN A VESICLE

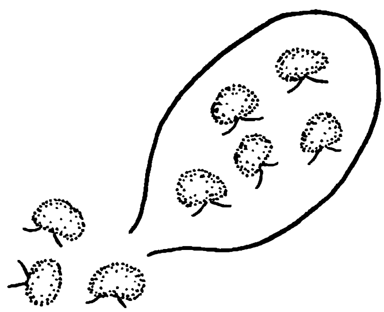


FIG. 19 DECIDUOUS SPORANGIUM

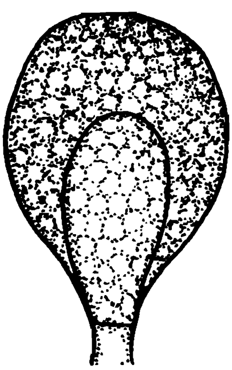


FIG. 20 SPORANGIUM WITH COLUMELLA

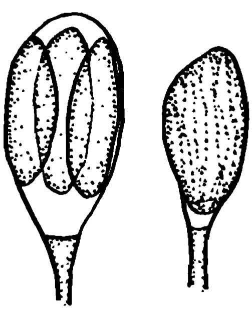


FIG. 21 SPORANGIOLES

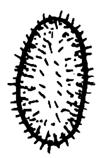


FIG. 22 CONIDIUM

Plate 4

CLASS OÖMYCETES

ASEXUAL REPRODUCTION

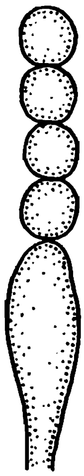


FIG. 23 SIMPLE SPORANGIOPHORE

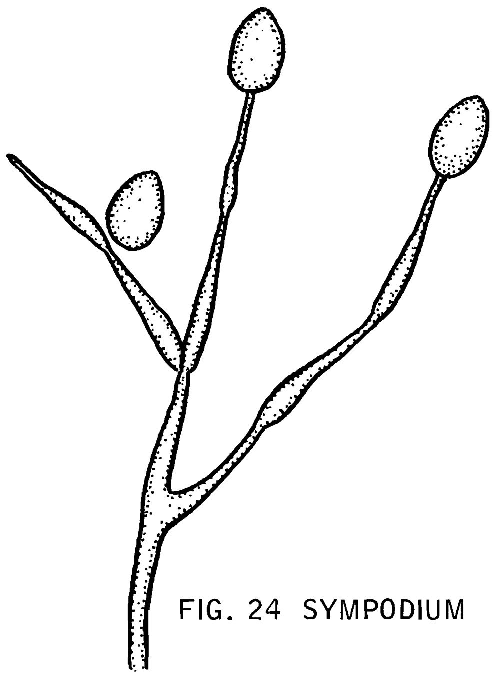


FIG. 24 SYMPODIUM

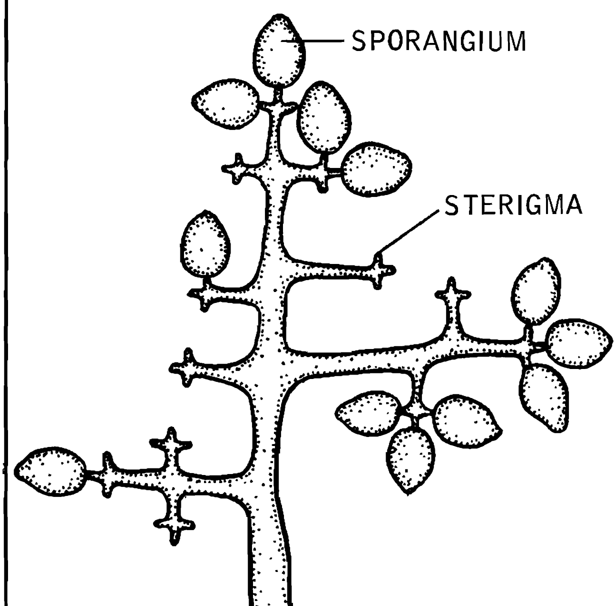


FIG. 25 MONOPODIUM

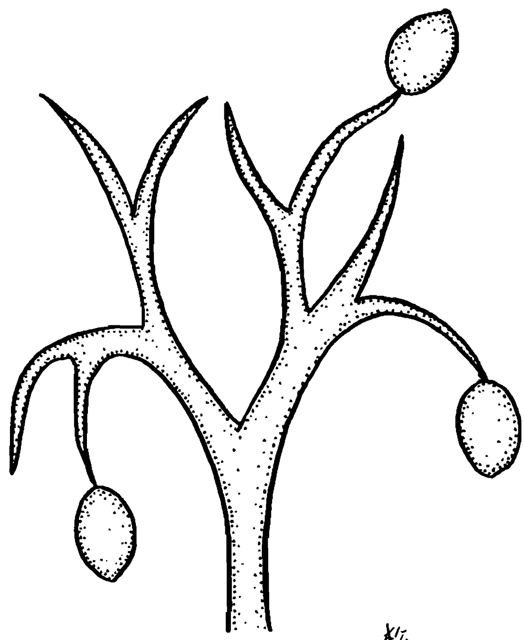


FIG. 26 DICHOTOMY

Plate 5

CLASSES CHYTRIDIOMYCETES (FIG. 27)
OOMYCETES (FIG. 28) ZYGOMYCETES (FIG. 29)

SEXUAL REPRODUCTION

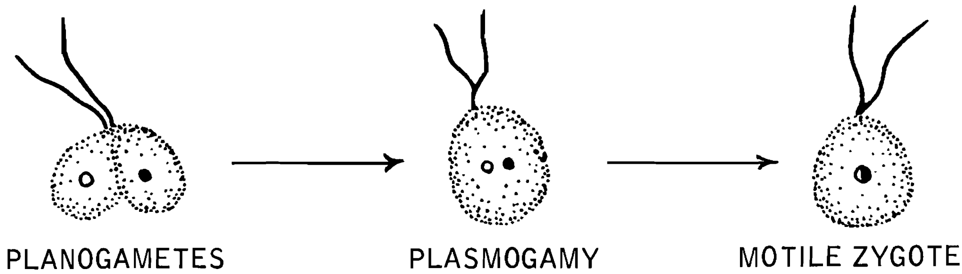


FIG. 27 PLANOGAMETIC COPULATION

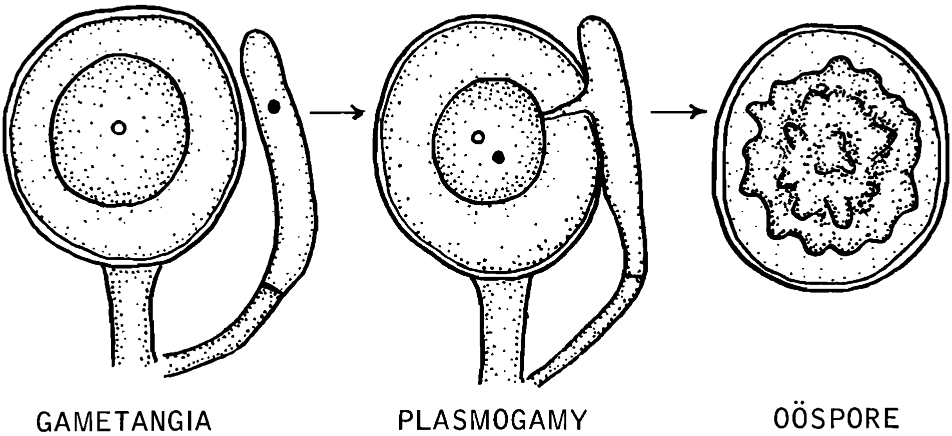


FIG. 28 GAMETANGIAL CONTACT

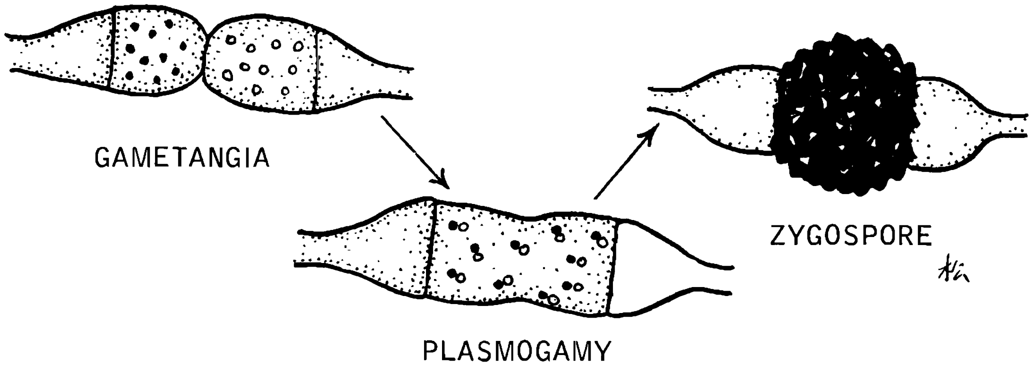


FIG. 29 GAMETANGIAL COPULATION

Plate 6

8. Search for the sporangia of the parasite which develop in the host hyphae. The zoöspores are discharged through one or more discharge papillae. Search your mount for zoöspores in the sporangia and for empty sporangia. The sporangial wall is fused with that of the host.

9. The resting spores are formed endobiotically in segments formed in the host hyphae. The wall is thick, reddish-brown in color and is covered with fine spines.

Family: Synchytriaceae

Genus: Synchytrium

10. Examine potato tubers infected with *Synchytrium endobioticum*. Note the effect of the infection on the host.

11. Study prepared slides of sections of infected tubers. Search the slide for the following stages in the life cycle of the parasite:

    a. Young prosorus
    b. Mature prosorus
    c. Germinating prosorus
    d. Sorus of sporangia
    e. Resting sporangium

    Observe the size of the nuclei in (a), (b), and (c). Note the relative thickness of the walls of the prosorus and the resting sporangium.

Family: Phlyctidiaceae

Genus: Rhizophidium

12. Mount and examine a species of *Rhizophidium* which is parasitizing the oögonia of *Saprolegnia*. Observe the rhizoidal system that extends into the oögonium of the host. Locate the external sporangia of the parasite and observe the lack of a lid (operculum) in the exit papillae. Search your slide for empty sporangia; if possible, observe the escape of the zoöspores from mature sporangia.

13. Smaller resting spores may be found near the sporangia. These have thicker walls.

Family: Physodermataceae

Genus: Physoderma

14. Examine infected corn leaves for sporangia of *Physoderma zea-maydis*. They appear as dark brown, globose or ovoid structures. Cut off a very small portion of the infected part of the leaf and boil for a few seconds in 10% KOH in a test tube. Mount in water under a cover glass and examine for sporangial sori. Study the sporangia under high power.

Family: Chytridiaceae

Genus: Chytridium

15. Mount some Oedogonium containing Chytridium olla, a species embodying most of the characteristics of a typical chytrid.

16. Observe the sessile, ovoid sporangium, the lid (operculum) which is usually umbonate. Locate the many-branched rhizoids extending within the host cells.

17. Look for thick walled-spores in the host cells. These are usually spherical and have a smooth wall.

Family: Megachytriaceae

Genus: Nowakowskiella

18. Mount a piece of cellophane, corn leaves or other substrates, containing Nowakowskiella elegans, on a slide and examine microscopically. Note the extensive, much branched rhizomycelium with irregular swellings.

19. Some of the swellings will form sporangia. Locate a sporangium before and after discharge of the zoöspores. Some of the empty sporangia may still bear a lid (operculum). New sporangia can usually be seen developing within the old ones, a process known as internal proliferation.

20. Some species of Nowakowskiella produce resting spores with thick walls. If these are present in your mount study the structures which you see.

C. QUESTIONS

1. What are the characteristics used to separate the first six classes of the subdivision Eumycotina? These have been grouped under the name Phycomycetes in previous references.
2. Characterize the class Chytridiomycetes.
3. What characteristics are used to subdivide the lower fungi or Phycomycetes into subdivisions or into the six classes by the two schools of classification as exemplified by Gäumann and Fitzpatrick on the one hand, and Sparrow and Bessey on the other.
4. Construct a life cycle diagram to illustrate the general life history pattern of one of the chytrids.
5. What determines the production of sporangia and gametangia in Synchytrium endobioticum?
6. What is the difference in effect upon the host of the haploid and the diploid infecting stages of this organism?
7. What are rhizoids? Rhizomycelium? How do they differ from true mycelium?

D. TEXT REFERENCES:

1. Alexopoulos -- pp. 100-117.
2. Bessey -- pp. 42-77.
3. Sparrow -- 42-603.

E. SELECTED REFERENCES

1. COUCH, J. N. 1941. The structure and action of the cilia in some aquatic Phycomycetes. Am. Jour. Bot., 28:704-713.
2. CURTIS, K. M. 1921. The life history and cytology of Synchytrium endobioticum (Schilb.) Perc., the cause of wart disease in potato. Phil. Trans. Roy. Soc. London, Ser. B., 210:409-478.
3. FITZPATRICK, H. M. 1930. The Lower Fungi. xi + 331 pp. McGraw-Hill Book Co., New York.
4. GÄUMANN, E. A. 1949. (English translation by F. L. Wynd, 1952.) The Fungi. Hafner Publishing Co., New York.
5. GOLDIE-SMITH, E. K. 1956. Maintenance of stock cultures of aquatic fungi. Jour. Elish. Mitch. Sci. Soc., 72:348-356.
6. KARLING, J. S. 1937. The cytology of the Chytridiales with special reference to Cladochytrium replicatum. Mem. Torrey Bot. Club., Vol. 19, No. 1. 92 pp.
7. KARLING, J. S. 1960. Synchytrium pilificum in America. Mycologia, 52:436-443.
8. KOCH, W. J. 1956. Studies of the motile cells of chytrids. I. Electron microscope observations of the flagellum, blepharoplast and rhizoplast. Amer. Bot., 43:811-819.
9. KOCH, W. J. 1958. Studies of the motile cells of chytrids. II. Internal structures of the body observed with light microscopy. Amer. Jr. Bot., 45:59-72.
10. MATTHEWS, Velma D. 1928. Nowakowskiella and a new species of Pythium. Jour. Elisha Mitchel Sci. Soc., 43:229-232.
11. SPARROW, F. K., JR. 1958. Interrelationships and phylogeny of the aquatic Phycomycetes. Mycologia 50:797-813.
12. SPARROW, F. K., JR. 1960. Aquatic Phycomycetes. xxv + 1187 pp. The University of Michigan Press. Ann Arbor.
13. UMPHLETT, C. J., and M. M. HOLLAND. 1960. Resting spores in Phyctochytrium planicorne. Mycologia, 52:429-435.
14. WILLOUGHBY, L. G. 1956. Studies on soil chytrids. 1. Rhizidium richmondense, sp. nov. and its parasites. Trans. Brit. Mycol. Soc., 39:125-141.
15. WILLOUGHBY, L. G. 1955. Inoculation studies with a polysporangiate Rozella parasitic in Dictyuchus anomalus. Amer. Jr. Bot., 42:119-123.

F. GLOSSARY:

1. Discharge papilla (L. papilla = a nipple): a nipple-like swelling on a sporangium which bursts and permits the zoöspores to escape.
2. Eucarpic (Gr. eu = good + karpos = fruit): forming reproductive structures on certain portions of the thallus, the thallus itself continuing to perform its somatic functions.
3. Holocarpic (Gr. holos = entirely + karpos = fruit): refers to an organism whose thallus is entirely converted into one or more reproductive structures.
4. Oögonium (pl. oögonia; Gr. oön = egg + gennao = I give birth): a female gametangium.
5. Operculum (pl. opercula; L. operculum = lid): a lid at the summit of a spore-bearing structure such as a sporangium or an ascus.
6. Prosorus (pl. prosori; Gr. pro = before + soros = heap): a structure which eventually divides and gives rise to a sorus.
7. Rhizoid (Gr. rhiza = root + oeides = like): a short, thin branch of a thallus, superificially resembling a root; an anchoring or absorbing organ or both.
8. Rhizomycelium (Gr. rhiza = root + mycelium): a rhizoidal system which is extensive enough to resemble mycelium.
9. Sorus (pl. sori; Gr. soros = heap): a mass of sporangia or spores.

Class Chytridiomycetes

Order: Blastocladiales

Family: Blastocladiaceae

A. GENERAL REMARKS:

1. The somatic structures of the Blastocladiales are eucarpic. The thallus consists of well-developed mycelium which is generally differentiated into a group of rhizoids, a trunk and branches. Asexual reproduction is by means of uniflagellate zoöspores formed in sporangia. Sexual reproduction, where known, is by the fusion of motile, uniflagellate isogametes or anisogametes. All genera also form thick-walled resistant sporangia which, after a dormant period, produce zoöspores. The cell walls usually give a reaction for chitin.

2. The order is divided into three families (Sparrow, 1960). The Coelomomycetaceae, lacking walls around the thallus and without rhizoids, are a specialized group of parasites usually on mosquito larvae. The Catenariaceae, with tubular, walled thallus, rhizoids and cross walls to delimit reproductive structures, are parasites of small worms or fungi and survive as saprophytes on various substrates. The Blastocladiaceae is the best known family, and is the only one we shall study.

3. The family Blastocladiaceae contains two distinct groups of fungi. One group, represented by Blastocladiella and Allomyces, has sporangia with several discharge pores and usually sexual reproduction accompanied by alternation of generations. Pseudosepta may be present. The second group, represented by Blastocladia, forms a single apical pore on the sporangium. The dichotomous branches lack pseudosepta and are limited in development. No sexual reproduction has been observed in Blastocladia.

B. PROCEDURE:

Genus: Blastocladiella

4. Examine fresh material, if available, in a water culture and in yeast-starch agar, of a species of Blastocladiella. Locate the basal rhizoids, the elongate unbranched basal cell, and the terminal single reproductive organ on the thallus. Mount some of the thalli in distilled water for observation microscopically. Use osmic acid-gentian violet, or Bouins fixative for observation of flagella (see page 3). Observe the action of flagella under the phase microscope.

5. Study the sporothalli. Locate the rhizoids, elongate basal cell, and either the thin-walled zoösporangia with one or more pores and the emergence of the uniflagellate zoöspores, or the thick-walled resting spores with brownish pigments.

6. Resting spores of some species (B. variabilis) develop gametothalli upon germination. If available, study the gametothalli of B. variablis. Note that the gametes are orange colored in some gametangia while others are colorless. Watch the two types of gametes for possible fusion to form a biflagellate zygote. These zygotes will, upon germination, develop the sporothallus.

Genus: Allomyces

7. The genus Allomyces is unique among the fungi in that the thallus is either gametophytic or sporophytic and thus exhibits a definite alternation of generations. The thallus is composed of rhizoids, a trunk, and successive dichotomous branches.

8. Examine water cultures of sporophytic thalli of Allomyces javanicus var. japonensis (or A. arbusculus). Examine the somatic structures and the two types of sporangia produced. Note the thick-walled resistant sporangia with pits on their walls. After a resting period, these sporangia give rise to zoöspores which form the gametophytic thalli. Thin-walled zoösporangia which also give rise to zoöspores are produced at the tips of the branches. These zoöspores develop into new sporophytic thalli. Look for zoöspores in the motile and in the germinating stage. Stain the flagella of the zoöspores.

9. Study gametophytic thalli which have developed from dry resistant sporangia. Note how the male and female gametangia differ from each other in size, color and position on the thallus.

10. Mount some of the gametophytic hyphae in distilled water. Observe gametangia which are liberating anisogametes. Note the difference in the size of the gametes and their method of movement. In a short time you may see the slightly larger gametes fusing with the smaller gametes to form zygotes. These zygotes are motile for a short period of time before germinating and producing sporophytic thalli.

Genus: Blastocladia

11. Examine prepared slides or, if fresh material is available, make a mount of a species of Blastocladia which grows as white pustules on the surface of submerged fruits of Crataegus, rose or other plant materials.

12. Observe the rhizoidal system, the main trunk, and the short dichotomous branches. Some branches are terminated by thin-walled sporangia which produce zoospores, while other branches contain thick-walled resistant sporangia. No sexual reproduction has been definitely established for the genus Blastocladia.

C. QUESTIONS

1. What is the normal habitat of the Blastocladiales?
2. How would you go about isolating members of this order?
3. Characterize the order Blastocladiales.
4. What is the significance of this group?
5. Diagram the life history of Allomyces (Euallomyces type).
6. In what respects does this life history differ from the typical pattern of the fungi?
7. How are the genera of the Blastocladiaceae distinguished?

D. TEXT REFERENCES:

1. Alexopoulos -- pp. 117-123.
2. Bessey -- pp. 78-86.
3. Sparrow -- pp. 604-695.

E. SELECTED REFERENCES:

1. BENEKE, E. S. and G. B. WILSON. 1950. Treatment of Allomyces javanicus var. japonensis Indoh with colchicine and sodium nucleate. Mycologia, 42:519-522.
2. BLACKWELL, E. 1940. A life cycle of Blastocladia pringsheimii Reinsch. Trans. Brit. Mycol. Soc., 24:68-96.
3. CANTINO, E. C. 1955. Physiology and phylogeny in the water molds - a reevaluation. Quart. Rev. Biol., 30:138-149.
4. CANTINO, E. C., and E. A. HORENSTEIN. 1956. Gamma and the cytoplasmic control of differentiation in Blastocladiella. Mycologia, 48:443-446.
5. CANTINO, E. C., J. LOVETT, and E. A. HORENSTEIN. 1957. Chitin synthesis and nitrogen metabolism during differentiation in Blastocladiella emersonii. Amer. Jr. Bot., 44:498-505.
6. EMERSON, R., and C. M. WILSON. 1954. Interspecific hybrids and the cytogenetics and cytotaxonomy of Euallomyces. Mycologia, 46:393-434.
7. EMERSON, RALPH. 1941. An experimental study of the life cycles and taxonomy of Allomyces. Lloydia, 4:77-144.
8. EMERSON, RALPH and E. C. CANTINO. 1948. The isolation, growth and metabolism of Blastocladia in pure culture. Am. Jr. Bot., 35:157-171.
9. HATCH, W. R. 1935. Gametogenesis in Allomyces arbuscula. Ann. Bot., 49:623-650.
10. HATCH, W. R. 1938. Conjugation and zygote germination in Allomyces arbuscula. Ann. Bot., n. s. 2:583-614.
11. INDOH, H. 1940. Studies on Japanese aquatic fungi II. The Blastocladiaceae. Sci. Reports Tokyo Bunrika Daigaku, Sec. B, 4:237-284.
12. KANOUSE, BESSIE B. 1925. On the distribution of the water molds with notes on the occurrence in Michigan of members of the Leptomitaceae and Blastocladiaceae. Papers Mich. Acad. Sci., 5:105-114.
13. KANOUSE, BESSIE B. 1927. A monographic study of special groups of the water molds. I. Blastocladiaceae. Am. Jr. Bot. 14:287-306.
14. MATTHEWS, V. D. 1937. A new genus of the Blastocladiaceae. Jour. Elisha Mitchell Sci. Soc., 53:191-195.
15. MC CRANIE, JAMES. 1942. Sexuality in Allomyces cystogenus. Mycologia, 34:209-213.
16. SPARROW, F. K., JR. 1960. Aquatic Phycomycetes. 2nd Ed. xxv + 1187 pp. University of Michigan Press. Ann Arbor.
17. WHIFFEN, ALMA J. 1951. The effect of cyclohexamide on the sporophyte of Allomyces arbuscula. Mycologia, 43:635-644.
18. WILSON, C. M. 1952. Meiosis in Allomyces. Bull Torrey Bot. Club, 79:139-160.
19. WOLF, F. T. 1941. A contribution to the life history and geographic distribution of the genus Allomyces. Mycologia, 31:376-387.

F. EDUCATIONAL FILM

Syngamy and alternation of generations in Allomyces - a water mold. Black and white. 20 min. Phase Films. Box 423, Ross, Calif.

G. GLOSSARY:

1. Anisogametes (Gr. an = not + isos = equal + gametes = husband): gametes which are similar in structure but which differ in size.
2. Gamete (Gr. gametes = husband): a differentiated sex cell or a sex nucleus which fuses with another in sexual reproduction.
3. Gametothallus (pl. gametothalli; Gr. gametes = husband + thallos = shoot): a a thallus which produces gametes, as opposed to a sporothallus.
4. Isogametes (Gr. isos = equal + gametes = husband): gametes which are similar in structure and in size and consequently morphologically indistinguishable.
5. Pseudoseptum (pl. pseudosepta; Gr. pseudo = false + L. septum = hedge, partition): a false septum.
6. Sporothallus (pl. sporothalli; Gr. sporos = seed, spore + thallos = shoot): a thallus which produces spores, as opposed to a gametothallus.
7. Zoösporangium (pl. zoösporangia; Gr. zoön = animal + sporangium) a sporangium which contains zoöspores.

Class Chytridiomycetes

Order: Monoblepharidales

Family: Monoblepharidaceae

A. GENERAL REMARKS:

1. This small group of eucarpic, aquatic fungi is of especial interest in that it is the only one possessing a large non-motile egg fertilized by a small motile sperm. The thallus consists of a well-developed mycelium. The hyphae are filled with a highly vacuolated protoplasm which gives a characteristic foamy appearance. Asexual reproduction is by sporangia containing uniflagellate zoöspores.

2. All of the Monoblepharidales are placed in a single family, the Monoblepharidaceae. This family includes three genera: Monoblepharis, Monoblepharella, and Gonapodya.

3. Representatives of two of these genera can usually be found in the temperate zones. Species of Monoblepharis can be found on dead, submerged twigs of ash, birch, and other tree species, in cool waters in the spring. Gonapodya occurs under very foul environmental conditions; it appears as white pustules on rosaceous fruits.

B. PROCEDURE:

Genus: Monoblepharis, Monoblepharella

4. Examine the somatic structures of a species of Monoblepharella growing on hemp seed. Note the delicate hyphae and the foamy appearance of the protoplasm. Observe the sporangia and the zoöspores escaping from the apical pore of the sporangium. If material is available, study the sexual reproductive structures.

5. Study the sexual reproduction of Monoblepharis polymorpha or any other species available. Note the position of the antheridia in relation to the oögonia. An oösphere can be seen in the unfertilized oögonium. After fertilization the oösphere changes into an oöspore. The fertilized oösphere emerges from the oögonium and, while still attached to the oögonial orifice, develops a thick wall with or without bullations.

Genus: Gonapodya

6. If material is available, study the somatic and asexual reproductive structures of Gonapodya. Note the constricted pseudosepta in the hyphae. The sporangia are terminal and more or less oval in shape. Young sporangia frequently develop by internal proliferation within old ones.

7. Study the sexual reproduction of Gonapodya polymorpha if present in the material. Note the larger female gametangium with the nonflagellated gametes and the male gametangium which contains uniflagellate zoöspores.

C. QUESTIONS

1. In what respects do the Monoblepharidales differ from the Chytridiales and the Blastocladiales? In what ways are the three orders similar?
2. How would you proceed to collect and isolate members of this order?
3. Construct a life cycle diagram of Monoblepharis polymorpha.

D. TEXT REFERENCES:

1. Alexopoulos -- pp. 123-126.
2. Bessey -- pp. 86-93.
3. Sparrow -- pp. 696-742.

E. SELECTED REFERENCES:

1. BENEKE, E. S. 1948. The Monoblepharidaceae as represented in Illinois. Trans. Ill. Acad. Sci., 41:27-31.
2. COKER, W. C. and VELMA D. MATTHEWS. 1937. Monoblepharidales. North Am. Flora, 2(1), 67 pp. New York Botanical Garden, New York.
3. JOHNS, R. M., and BENJAMIN, R. K. 1954. Sexual reproduction in Gonapodya. Mycologia, 46:201-208.
4. SHANOR, L. 1942. A new Monoblepharella from Mexico. Mycologia, 34:241-247.
5. SPARROW, F. K., JR. 1933. The Monoblepharidales. Ann. Bot., 47:517-542.
6. SPARROW, F. K., JR. 1953a. A new species of Monoblepharella. Mycologia, 45:592-595.
7. SPARROW, F. K., JR. 1953b. Cytological observations on the zygote of Monoblepharella. Mycologia, 45:723-726.
8. SPARROW, F. K., JR. 1960. Aquatic Phycomycetes. 2nd Ed. xxv + 1187 pp. University of Michigan Press. Ann Arbor.
9. SPRINGER, MARTHA E. 1945. A morphologic study of the genus Monoblephaella. Am. Jr. Bot., 32:259-269.

F. GLOSSARY:

1. Antheridium (pl. antheridia; Gr. antheros = flowery + idion, a dimin. suffix): a male gametangium.
2. Oösphere (Gr. oön = egg + sphaira = sphere): a large, naked, non-motile, female gamete.
3. Oöspore (Gr. oön = egg + sporos = seed, spore): a thick-walled spore which develops from an oösphere either through fertilization or parthenogenesis.

Class Hyphochytridiomycetes

A. GENERAL REMARKS:

1. The class Hyphochytridiomycetes contains organisms that produce anteriorly uniflagellate zoöspores with tinsel type flagella. Varied types of thalli are found in this group. Only one known incidence of sexual reproduction is known.

2. This class includes one order: the Hyphochytriales.

Order: Hyphochytriales

A. GENERAL REMARKS:

3. The Hyphochytriales are aquatic fungi resembling the Chytridiales except for the tinsel type anteriorly uniflagellate zoöspores. They are inoperculate, holocarpic or eucarpic, with the somatic phase rhizoidal or hypha-like, containing intercalary swellings.

4. This order contains three families: the Rhizidiomycetaceae, the Anisolpidiaceae, and the Hyphochytriaceae. Only one of the families will be discussed below.

B. PROCEDURE:

Family: Rhizidiomycetaceae

Genus: Rhizidiomyces

5. Examine some oögonia and oöspores of members of the Saprolegniales, or algae parasitized by Rhizidiomyces sp., or a pure culture of the organism. Locate eucarpic thallus with many branched rhizoids and the sporangium. Observe the sporangial discharge tube and the vesicle with the differentiation of zoöspores.

6. Observe the anteriorly uniflagellate zoöspores under a phase microscope or after the zoöspores have been stained.

C. QUESTIONS:

1. Compare the similarities and differences between the Chytridiomycetes and the Hyphochytridiomycetes.

D. TEXT REFERENCES:

Alexopoulos -- pp. 130-133.
Bessey -- pp. 69-71.
Sparrow -- pp. 743-767.

E. SELECTED REFERENCES:

1. COKER, W. C. 1923. The Saprolegniaceae, with notes on other water molds. 201 pp. Univ. North Carolina Press. Chapel Hill.

2. FULLER, M. S. 1960. Biochemical and microchemical study of the cell walls of Rhizidiomyces sp. Amer. Jr. Bot., 47:105-109.
3. FULLER, M. S. 1962. Growth and development of the water mold Rhizidiomyces in pure culture. Amer. Jr. Bot., 49:64-71.
4. KARLING, J. S. 1939. A new fungus with anteriorly uniciliate zoöspores: Hyphochytrium catenoides. Amer. Jr. Bot., 26:512-519.
5. SPARROW, F. K., JR. 1960. Aquatic Phycomycetes. xxv + 1187 pp. University of Michigan Press. Ann Arbor.
6. ZOPF, W. 1884. Zur Kenntniss der Phycomyceten. I. Zur Morphologie und Biologie der Ancylisteen und Chytridiaceen. Nova Acta Acad. Leop.-Carol., 47:143-236.

F. GLOSSARY:

1. Vesicle (L. vesicula = small bladder): a bladder-like sac; in the aquatic fungi, a thin, bubble-like structure in which zoöspores are released or in which zoöspores are differentiated.

Class Oömycetes

A. GENERAL REMARKS:

1. In the Oömycetes, all of the orders have biflagellate zoöspores with oppositely directed, nearly equal, flagella. The forwardly directed flagellum is of the tinsel type, the posteriorly directed one is of the whiplash type. The mycelium is well-developed in most cases, coenocytic, non-septate, and well-branched. Sexual reproduction results in the formation of a resting spore or spores of the oögamous type.

2. This class includes four orders: the Lagenidiales, the Saprolegniales, the Leptomitales, and the Peronosporales.

Order: Lagenidiales

A. GENERAL REMARKS:

3. The Lagenidiales are aquatic fungi which are usually parasitic in fresh water algae and in various aquatic fungi. The somatic structures are holocarpic, endobiotic, with simple or multicellular thalli. Asexual reproduction is by means of laterally biflagellate zoöspores produced in a sporangium with one or more discharge papillae. Resting spores may be formed asexually or sexually after the copulation of the receptive thallus with one or more other thalli.

4. The order has been tentatively subdivided into three families by Sparrow. One family, the Olpidiopsidaceae, has been considered in the past as allied to the Chytridiales, but it appears to be more closely related to the family Lagenidiaceae. The third family, Sirolpidiaceae, consists entirely of marine fungi on green and red seaweeds. We shall consider only the first two families.

5. The thallus of the Olpidiopsidaceae is always one-celled. The entire thallus is converted into a sporangium, which forms zoöspores, or into a gametangium, which copulates with another. The resting spore, which results from fertilization, remains free in the host in which the parasite lives and reproduces.

6. In contrast to the Olpidiopsidaceae, the Lagenidiaceae have multicellular thalli. The zoöspores are released from a vesicle which forms at the apex of the discharge papilla of the sporangium. The resting spore is formed with a gametangium.

B. PROCEDURE:

Family: Olpidiopsidaceae

Genus: Olpidiopsis

7. Examine a culture of Achlya biflagellata that is well parasitized by Olpidiopsis varians. Note how the host hyphae become greatly swollen.

8. Mount a few infected hyphae of Achlya and examine the swollen regions. Note that smooth-walled sporangia and spiny-walled sporangia are produced by the

same organism. This, however, is not true of all species of Olpidiopsis. Look for the discharge papillae and for the sporangia that are empty or are in the process of discharging zoöspores.

9. Look for resting spores of Olpidiopsis which may have been formed within the infected hyphae of the host. These develop from the receptive thallus (oögonium) after it has copulated with one or more contributing thalli (antheridia or companion cells). Locate a mature, thick-walled, spiny resting spore and the empty adjoining cell or cells.

10. If other genera of the Olpidiopsidaceae are available, study and compare their structures with those of Olpidiopsis.

Family: Lagenidiaceae

Genus: Lagenidium

11. Examine a species of Lagenidium which has parasitized one of the freshwater algae. Compare the thallus, the sporangia, and the sex organs and resting spores with those of Olpidiopsis.

C. QUESTIONS:

1. Characterize the order Lagenidiales.
2. Construct a life cycle diagram for Olpidiopsis; for Lagenidium.
3. Explain why Lagenidium and related organisms were removed from the order Ancylistales and placed in a newly established order Lagenidiales.
4. Discuss the taxonomic position of Olpidiopsis. Defend Gäumann's view; Sparrow's view.
5. Discuss the phylogenetic and taxonomic significance of the flagellum of the lower fungi.

D. TEXT REFERENCES:

Alexopoulos -- pp. 134-138.
Bessey -- pp. 94-104.
Sparrow -- pp. 904-1011.

E. SELECTED REFERENCES:

1. BERDAN, HELEN. 1938. Revision of the genus Ancylistes. Mycologia, 30:396-415.
2. COUCH, J. N. 1935. A new saprophytic species of Lagenidium, with notes on other forms. Mycologia, 27:376-387.
3. GÄUMANN, E. A. 1949. (English translation by F. L. Wynd, 1952.) The Fungi. Hafner Publishing Co., New York.
4. MC LARTY, D. A. 1941. Studies in the family Woroninaceae. I. Discussion of a new species including a consideration of the genera Pseudolpidium and Olpidiopsis. Bull. Torrey Bot. Club, 68:49-66.
5. SHANOR, L. 1939. Studies in the genus Olpidiopsis. I. Resting spore germination in a new species. Jour. Elisha Mitchell Sci. Soc., 55:167-177.
6. SHANOR, L. 1939. Studies in the genus Olpidiopsis. II. The relationship of Pseudolpidium Fischer and Olpidiopsis (Cornu) Fischer Jour. Elisha Mitchell Sci. Soc., 55:179-195.
7. SPARROW, F. K., JR. 1960. Aquatic Phycomycetes. 2nd Ed. xxv + 1187 pp. University of Michigan Press, Ann Arbor.
8. VISHNIAC, H. S. 1955. The morphology and nutrition of a new species of Sirolpidium. Mycologia, 47:633-645.

F. GLOSSARY:

1. Endobiotic (Gr. endos = inside + bios = life): living inside a host; an organism which completes its entire life cycle inside its host.

Class Oömycetes

Order: Saprolegniales

A. GENERAL REMARKS:

1. The Saprolegniales include many aquatic or soil fungi in which the mycelium is usually well-developed and eucarpic. However, as mentioned below, some simple holocarpic forms are also classified in this order. Asexual reproduction takes place by means of biflagellate zoöspores or aplanospores which are formed in sporangia. The species in this order are diplanetic, monoplanetic or aplanetic, and some genera exhibit repeated zoöspore emergence. Sexual reproduction is accomplished by means of gametangial contact resulting in the formation of oöspores. All of the oögonial protoplasm is used in the formation of one or more oöspheres.

2. In more recent classifications the Saprolegniales have been expanded to include three families: the Ectrogellaceae, the Thraustochytriaceae and the Saprolegniaceae. The Ectrogellaceae include simple, holocarpic fungi that are chytrid-like. They develop inside fresh water and marine diatoms and certain brown algae. The Thraustochytriaceae contain but a single genus, Thraustochytrium, which is similar to the chytridiaceous genus Rhizophidium, but has biflagellate zoöspores which are released in a Thraustotheca-like manner. The Thraustochytriaceae are saprobic on marine algae. These families are described in more detail by Sparrow (1960).

3. The family Saprolegniaceae, the most typical of the order, includes the "water molds" that are frequently found as saprobes in fresh water and soil. A few are parasitic on fish and some parasitize the roots of certain plants. The Saprolegniaceae are eucarpic, with unlimited growth in the mycelium. The behavior of the spores, which is an important characteristic in separating the genera, varies from diplanetic to aplanetic.

B. PROCEDURE:

Family: Saprolegniaceae

4. Collect some twigs, pieces of leaves, dead insects, dead fish, and submerged soil in slow moving streams, ponds, or lakes and place each of the different types of material collected in separate Petri dishes. Add water from the source of the materials or sterile distilled water to the Petri dishes so that the materials are covered. Add halves of hemp seeds or other seeds, or dead insects to the cultures. After 2 to 4 days observe the twigs, etc. for presence of hyphal growth. The genera of the Saprolegniaceae are determined on the basis of the manner of zoöspore discharge. See if you can determine any of the genera that develop.

5. Start water cultures of Saprolegnia sp., and other genera by cutting out 1/4 inch blocks of agar (maltose-peptone agar) cultures and removing from the Petri dish. Place the block in a sterile Petri dish and nearly cover with sterile distilled water. Place a half of a hemp seed facing downward on top of the agar block. After one day at room temperature, cover the seed with additional water. In

2 to 3 days colonies of the aquatic fungus will develop and reproduce asexually. Usually in about 5 to 7 days sexual reproduction may be observed.

Genus: Saprolegnia

6. Examine under the low power objective a culture of Saprolegnia ferax or other species growing on half of a hemp seed in water. Study the somatic hyphae, the oögonia, the antheridia and the sporangia. Look for zoöspores, encysted zoöspores, and germinating spores with germ tubes. If time permits watch the emergence of the zoöspores from the sporangia. This can be observed better by mounting a mature sporangium in distilled water and covering with a cover glass.

7. Mount a small portion of the thallus in distilled water. Study the hyphae which bear sporangia at their tips. It is possible to observe the formation and release of zoöspores in a sporangium at the right stage of development. Study the movement of the zoöspores and the germination of encysted zoöspores under the high power or oil immersion objective. In this genus new sporangia form inside the old empty ones, a development known as internal proliferation.

8. Remove a drop of water from the Petri dish containing zoöspores. Mount and observe under the phase contrast microscope, or use a flagella stain (see directions in the introduction). Determine the location of the flagella on the zoöspores.

9. Study hyphae which bear young and old sex organs. Look for young oögonia with antheridial branches appressed to the oögonial wall. Find some older oögonia in which the protoplasts have differentiated into oöspheres. Count the number of oöspheres in several oögonia. Find some intercallary as well as terminal oögonia. The oöspheres change to oöspores after they are fertilized or parthenogenetically in the absence of fertilization. The oöspores contain oil droplets in a centric arrangement.

Genus: Achlya

10. Examine a culture of Achlya following the same directions as given for Saprolegnia. Note that zoöspores have the first swimming stage largely suppressed and encyst at the mouth of the sporangium. Some species have oil droplets in a concentric arrangement in the oöspores. The arrangement of the oil droplets seems to be a specific character.

Genera: Pythiopsis, Isoachlya, Leptolegnia, Protoachlya, Aphanomyces, Calyptralegnia, Thraustotheca, Dictyuchus, Brevilegnia, Geolegnia.

11. If cultures of any of the above genera are available, study the zoöspore behavior, variations in the sporangia, and variations in the oögonia. In some of these genera the zoöspores encyst within the sporangia and the swimming stage may be entirely lacking.

C. QUESTIONS:

1. Characterize the Saprolegniales.
2. How would you proceed to isolate members of the Saprolegniaceae?
3. What is the economic importance of the Saprolegniaceae?

4. What factors control sexual reproduction in the Saprolegniaceae?
5. Discuss the probable evolution of asexual reproduction in the Saprolegniaceae.
6. Explain the difference between: aplanetism, monoplanetism, diplanetism, and repeated zoöspore emergence.
7. Construct a key to the genera of the Saprolegniaceae.
8. What is the relationship of hormones to stages of sexual reproduction?

D. TEXT REFERENCES:

Alexopoulos -- pp. 138-149.
Bessey -- pp. 104-116.
Sparrow -- pp. 792-851.

E. SELECTED REFERENCES:

1. BHARGAVA, K. S. 1950. Cytological studies of some members of the family Saprolegniaceae I. The vacuome. Cytologia, 16:72-83.
2. BHARGAVA, K. S. 1951. Cytological studies of some members of the family Saprolegniaceae II. The chondriome. Cytologia, 16:84-94.
3. COKER, W. C. 1923. The Saprolegniaceae, with notes on other water molds. 201 pp. University of North Carolina Press.
4. COKER, W. C. and VELMA D. MATTHEWS. 1937. Saprolegniales (Saprolegniaceae, Ectrogellaceae, Leptomitaceae). North Am. Flora, 2(1):15-67.
5. COOK, W. R. I. and ENID MORGAN. 1934. Some observations on the Saprolegniaceae of the soils of Wales. Jour. Bot., 72:345-349.
6. COUCH, J. N. 1926. Heterothallism in Dictyuchus, a genus of the water molds. Ann. Bot., 38:309-361.
7. DRECHSLER, C. 1929. The beet water mold and several related root parasites. Jour. Agr. Res., 38:309-361.
8. KENDRICK, J. B. 1927. The black-root disease of radish. Indiana Agr. Exp. Station Bull., 311. 32 pp.
9. MANTON, I., B. CLARKE, and A. D. GREENWOOD. 1951. Observations with the electron microscope on a species of Saprolegnia. Jour. Exp. Bot., 2:321-331.
10. RAPER, J. R. 1936. Heterothallism and sterility in Achlya and observations on the cytology of Achlya bisexualis. Jour. Elisha Mitchell Sci. Soc., 52:274-289.
11. RAPER, J. R. 1940. Sexuality in Achlya ambisexualis. Mycologia, 32:710-727.
12. RAPER, J. R. 1951. Sexual hormones in Achlya. American Scientist, 39(1):110-120; 130.
13. SALVIN, S. B. 1942. Factors controlling sporangial type in Thraustotheca primoachlya and Dictyuchus achlyoides. I. Am. Jour. Bot., 29:97-104.
14. SHANOR, L. 1937. Observations on the development and cytology of the sexual organs of Thraustotheca clavata (deBary) Humphr. Jour. Elisha Mitchell Sci. Soc., 53:119-135.
15. SPARROW, F. K., JR. 1960. Aquatic Phycomycetes. 2nd Ed. xxv + 1187 pp. University of Michigan Press, Ann Arbor.
16. TIFFNEY, W. N. 1939. The host range of Saprolegnia parasitica. Mycologia, 31:310-321.
17. WHIFFEN, A. J. 1960. Interthallic sexual reactions in Achlya, a genus of the aquatic fungi. Mycologia, 47:14-23.
18. WOLF, F. T. 1944. The aquatic Oömycetes of Wisconsin. 64 pp. The University of Wisconsin Press.

F. GLOSSARY:

1. Aplanetic (Gr. a = not + planetes = wanderer): non-motile.
2. Diplanetic (Gr. dis = twice + planaomai = I wander): refers to a species which produces two types of zoöspores and in which two swarming periods occur.
3. Encystment (Gr. en = inside _ kystis = bladder): formation of a cell wall around the naked protoplast.
4. Monoplanetic (Gr. monos = alone + planaomai = I wander): refers to a species which produces only one type of zoöspores and in which there is but one swarming period.

Class Oömycetes

Order: Leptomitales

A. GENERAL REMARKS:

1. This is a small order of aquatic fungi with the mycelium constricted at regular intervals by pseudosepta of cellulin. Monoplanetic or diplanetic zoöspores are formed in sporangia that are either elongated or short and pyriform. Sexual reproduction is by gametangial contact. A single oösphere is formed in the oögonium. Periplasm may be present around the oösphere. A single segment of the hypha may function as the antheridium or special branches may develop.

2. The Leptomitales contain two recognized families: the Leptomitaceae and the Rhipidiaceae. The Leptomitaceae have a thallus entirely filamentous throughout; they produce diplanetic zoöspores; and they lack any differentiation of periplasm in the oögonium. The Rhipidiaceae are distinguished from the Leptomitaceae by a differentiation of the thallus into holdfasts, basal cell, and hyphal branches; their zoöspores are monoplanetic; and the oögonium has periplasm.

3. The Leptomitales are saprobic on vegetable debris, especially twigs and fruits that are submerged in water. One genus, Leptomitus, may be found in heavily polluted water.

B. PROCEDURE:

Family: Leptomitaceae

Genus: Apodachlya

4. Examine a culture of *Apodachlya pyrifera* (or other species) growing on half of a hemp seed in distilled water. Note the slimy appearance of the culture.

5. Cut off a portion of the mycelium and mount. Note the characteristic structure of the hyphae which have one or two cellulin plugs in each segment. Study the shape of the sporangia, the zoöspores, the encysted spores, and the germinating spores.

6. Locate the spherical oögonia which are borne terminally or on short lateral segments. The antheridium may be seen as a short lateral outgrowth below the oögonium. Locate a mature oöspore with one or two oil globules.

Genus: Leptomitus

7. If living material of *Leptomitus* is available, compare this genus with *Apodachlya*. Note that the mycelium is larger, the sporangia are formed in undifferentiated segments of the hyphae, and sexual reproduction is apparently lacking.

Family: Rhipidiaceae

Genera: Araiospora, Sapromyces, Rhipidium, and Mindeniella

8. If preserved or living material is available for any of the genera in this family, note that the thallus is differentiated into a holdfast, basal cell, and hyphal branches. The sporangial wall is smooth in some and spiny in others. Observe an oögonium with oöplasm and periplasm.

C. QUESTIONS:

1. What is the difference between the Saprolegniales and the Leptomitales?
2. What evolutionary advances are represented in the Leptomitales over the Saprolegniales?
3. How would you proceed to collect and isolate members of the Leptomitales?

D. TEXT REFERENCES:

Alexopoulos -- pp. 149-150
Bessey -- pp. 116-119.
Sparrow -- pp. 852-903.

E. SELECTED REFERENCES:

1. BISHOP, H. 1940. A study of sexuality on Sapromyces Reinschii. Mycologia, 32:505-529.
2. COKER, W. C. 1923. The Saprolegniaceae, with notes on other water molds. 201 pp. University of North Carolina Press. Chapel Hill.
3. INDOH, H. 1939. Studies on Japanese aquatic fungi. I. On Apodachlya completa, sp. nov., with revision of the Leptomitaceae. Sci. Repts. Tokyo Bunrika Daigaku, B. 4:43-50.
4. KANOUSE, BESSIE B. 1927. A monographic study of special groups of the water molds. II. Leptomitaceae and Pythiomorphaceae. Am. Jour. Bot., 14:335-357.
5. KEVORKIAN, A. G. 1935. Studies in the Leptomitaceae. II. Cytology of Apodachlya brachynema and Sapromyces Reinschii. Mycologia, 27:274-285.
6. SHADE, A. L. 1940. The nutrition of Leptomitus. Am. Jour. Bot., 27:376-384.
7. SHANOR, L. and L. S. OLIVE. 1942. Notes on Araiospora streptandra. Mycologia, 34:536-542.
8. SPARROW, F. K., JR. 1960. Aquatic Phycomycetes. 2nd Ed. xxv + 1187 pp. University of Michigan Press. Ann Arbor.

F. GLOSSARY:

1. Holdfast: an anchoring cell or system of rhizoids.
2. Periplasm (Gr. peri = around + plasma = something molded): a protoplasmic layer which surrounds the oösphere within the oögonium.

Class Oömycetes

Order: Peronosporales

Family: Pythiaceae

A. GENERAL REMARKS:

1. The Peronosporales constitute the most advanced order of the biflagellate Oömycetes. Among them are aquatic, amphibious and terrestrial forms, many of them destructive parasites of economic plants. The most highly advanced species are obligate parasites of Angiosperms. The mycelium is well-developed and eucarpic in all species in this order. The sporangia remain attached to the hyphae in the lower forms, but are deciduous in the higher forms. Biflagellate, reniform zoöspores are produced as a rule, but in many of the most advanced types, the sporangium behaves as a spore and germinates directly by a germ tube. Sexual reproduction is by gametangial contact resulting in the formation of an oöspore. A single oösphere surrounded by periplasm is usually produced by each oögonium.

2. The Peronosporales are subdivided into three families by most authors. These are: the Pythiaceae, the Peronosporaceae, and the Albuginaceae.

3. The family Pythiaceae represents the most primitive group of the Peronosporales. Many of them resemble the Saprolegniales to such an extent that some authors classify them in that order instead of in the Peronosporales.

4. In the lower members of the Pythiaceae the sporangia are produced at the tips of the somatic hyphae. In the higher members, the sporangia are borne at the tips of special reproductive hyphae, the sporangiophores. If sporangiophores are present, they are of indeterminate growth and form a sympodium.

B. PROCEDURE:

Genus: Zoöphagus

5. If material is available, study Zoöphagus insidians. This organism may be found in water cultures with twigs. Observe the branching hyphae connected to the twigs and ramifying between and upon algae in the culture. Note the peg-like or spine-like lateral branches on the hyphae. These are the modified branches which capture moving rotifers. Zoösporangia are similar to the mycelium with zoöspores usually developing at the tips in a vesicle. Oögonia are spherical, terminating a lateral branch, and contain one oöspore. An antheridium may be seen on the oögonium.

Genus: Pythium

6. Examine a culture of Pythium aphanidermatum or other species growing on hemp seed in water. Use the low power objective, and placing the Petri dish, with the colony in it, on the stage of the microscope, look for sporangia at the tips of the somatic hyphae. The sporangia, full of granular protoplasm, may appear brownish by transmitted light.

7. Sporangia release zoöspores in transparent bubble-like vesicles. The zoöspores in a vesicle are very active. Search your colony for a vesicle; watch it until it bursts and releases the zoöspores. If the colony is of the right age, you should be able to follow the development of the vesicle and the maturation of the zoöspores in a water mount from the culture.

8. Prepare a water mount of a small portion of the colony under a cover glass. Be sure the hyphae are well separated before the cover glass is applied. Study the somatic hyphae.

9. Study the sporangia and their contents under high power. Note that the sporangia remain attached to the mycelium even after the zoöspores are released.

10. Examine your mount for oögonia, antheridia, and oöspores. Usually one oöspore is present in each oögonium. The unfertilized oösphere may or may not have conspicuous periplasm. The antheridium or several antheridia are attached on the side of the oögonial wall. Note the fertilization tube.

Genus: Phytophthora

11. Examine potato leaves which have been parasitized by Phytophthora infestans. Cut a portion of an infected part of the leaf and place it in a test tube with about 3 to 4 mls. of a 10% solution of KOH. Heat the liquid almost to a boil to render it somewhat transparent. Place the material on a slide with the lower epidermis of the leaf facing up. Examine under low power.

12. Look for sporangiophores emerging from the stomata of the leaves. Study a stoma and surrounding epidermal cells; find sporangiophores, if possible with attached sporangia.

13. Scrape some sporangiophores from fresh or dried infected leaves. Mount in lacto-phenol, and study under high power. Note the sympodial branching of the sporangiophore, and the lemon-shaped sporangia.

14. If fresh material is available, place a large drop of distilled water on each of two clean glass slides and, using a camel's hair brush, brush off some sporangia from infected leaves or potato tubers onto the water drops. Place each slide in a moist chamber and incubate one in the refrigerator at about 10° C. and the other in an incubator at about 24° C. Observe at intervals beginning after 3 hours, and note the method of germination at each temperature.

15. Study other species of Phytophthora which are made available to you. Observe the sporangia, the sexual organs, and the oöspores.

C. QUESTIONS:

1. Characterize the Pythiaceae from the standpoint of somatic structures, asexual reproduction, and sexual reproduction.
2. Some authors classify the Pythiaceae in the Saprolegniales; give reasons for and against this viewpoint.
3. Distinguish between Pythium and Phytophthora.
4. Name some important plant diseases caused by each of these two genera and give the name of the causal organism for each disease.
5. By the use of diagrams illustrate the life-cycle of Pythium debaryanum; of Phytophthora infestans.
6. Discuss sporangial germination in the Pythiaceae.

D. TEXT REFERENCES:

Alexopoulos -- pp. 151-162.
Bessey -- pp. 126-135.
Sparrow -- pp. 1012-1060.

E. SELECTED REFERENCES:

1. BLACKWELL, ELIZABETH. 1943. The life history of Phytophthora cactorum (Leb. and Cohn) Schroet. Trans. Brit. Mycol. Soc., 26:71-89.
2. DRECHLER, C. 1947. Germination of Oospores of Pythium Butleri and Pythium tardicrescens. Phytopath. 37:438-439.
3. FITZPATRICK, H. M. 1930. The lower fungi. Phycomyetes. xi + 331 pp. McGraw-Hill Book Co. New York.
4. GALINDO, J. A. and M. E. GALLEGLY. 1960. The nature of sexuality in Phytophthora infestans. Phytopath., 50:123-128.
5. GALLEGLY, M. E. and J. A. GALINDO. 1958. Mating types and oöspores of Phytophthora infestans in nature in Mexico. Phytopath., 48:274-277.
6. LARGE, E. C. 1940. The advance of the fungi. 488 pp. Henry Holt and Co., New York.
7. MATTHEWS, VELMA D. 1931. Studies on the genus Pythium. 136 pp. University of North Carolina Press. Chapel Hill.
8. MEHROTRA, B. S. 1951. Physiological studies of some Phytophthoras. III. Carbon requirements. Lloydia, 14: 122-128.
9. MIYAKE, K. 1901. The fertilization of Pythium deBaryanum. Ann. Bot., 15:653-667.
10. MIDDLETON, J. T. 1943. The taxonomy, host range and geographic distribution of the genus Pythium. Mem. Torrey Bot. Club., 20:1-171.
11. MUNDKUR, B. D. 1949. Morphology and cytology of development of the sex organs of Phytophthora himalayensis Dastur. Bot. Gaz., 110:475-486.
12. MURPHY, P. A. 1927. The production of the resting spore of Phytophthora infestans on potato tubers. Sci. Proc. Roy. Soc. Dublin, 18:407-412.
13. NIEDERHAUSER, J. S. 1956. The blight, the blighter, and the blighted. Trans. N. Y. Acad. Sci., 19:55-63.
14. PROWSE, G. A. 1954. Sommerstorffia spinosa and Zoophagus insidians predaceous on rotifers, and Rozellopsis inflata the endoparasite of Zoophagus. Trans. Brit. Mycol. Soc., 34:400-405.
15. SPARROW, F. J., JR. 1960. Aquatic Phycomycetes. xxv + 1187. The University of Michigan Press. Ann Arbor.
16. TUCKER, C. M. 1931. Taxonomy of the genus Phytophthora de Bary. Mo. Agr. Exp. Sta. Bull., 153. 208 pp.
17. WATERHOUSE, G. M. 1956. The genus Phytophthora. Diagnoses (or descriptions) and figures from the original papers. Misc. Publ. Commonw. Mycol. Inst. 12. 120 pp.

F. GLOSSARY:

1. Sporangiophore (Gr. sporos = seed, spore + angeion = vessel + phoreus = bearer): a specialized reproductive hypha which bears sporangia.

Class Oömycetes

Order: Peronosporales

Family: Peronosporaceae

A. GENERAL REMARKS:

1. The Peronosporaceae are considered the most advanced family of the biflagellate Oömycetes. All of them are obligate parasites and many cause destructive diseases of economic plants. Diseases caused by members of this family are called "downy mildews".

2. The most common genera of the Peronosporaceae are: Plasmopara, Peronospora, Basidiophora, Sclerospora, Bremia, and Pseudoperonospora. They are distinguished on the basis of sporangiophore structure and mode of branching.

B. PROCEDURE:

Genus: Plasmopara

3. Examine grape leaves and berries which have been infected by Plasmopara viticola. Note the downy growth on the underside of the leaves and on the surface of the berries. This consists of sporangiophores and sporangia, which emerge through the stomata.

4. Carefully scrape some of this downy growth on a slide and mount under a cover glass. Find a typical sporangiophore with attached sporangia. Be sure to understand the method of branching of the sporangiophore. This is monopodial branching and is typical of the genus Plasmopara. Note the tiny prongs on which the sporangia are borne. These are the sterigmata.

5. Examine prepared slides showing sections of infected grape leaves. Find the hyphae and the haustoria of the parasite. The hyphae are intercellular whereas the haustoria are food-absorbing organs which the hyphae sink into the host cells. Study the haustoria under the oil immersion objective. The Peronosporaceae produce many different kinds of haustoria; this is just one type.

Genus: Peronospora

6. Examine oösporic material of this fungus or of any of the other genera of the Peronosporaceae. Note the thick Oöspore wall.

7. Examine leaves of a plant infected with a species of Peronospora. Note the downy growth on the underside of the leaves, which consists of sporangiophores bearing sporangia.

8. Mount some sporangiophores on a slide under a cover glass. Note the type of branching. This is dichotomous branching and is typical of the genus Peronospora. Note the graceful branches ending in pointed tips as contrasted to the awkward sporangiophores of Plasmopara.

C. QUESTIONS:

1. In tabular form compare and contrast the Peronosporaceae and the Pythiaceae.
2. Which of the families in (1) is the most advanced? Why?
3. How do the sporangia of *Plasmopara viticola* germinate? Those of *Peronospora*?
4. Would it be correct to call the sporangia of the Peronosporaceae "conidia"? Explain.
5. Distinguish between sympodial, monopodial, and dichotomous branching.
6. Diagram the life history of *Plasmopara viticola*.
7. Construct a key to the genera of the Peronosporaceae.
8. Name 5 important plant diseases caused by members of the Peronosporaceae and give the name of the causal agent for each disease.
9. Of what historic significance is *Plasmopara viticola*?

D. TEXT REFERENCES:

Alexopoulos -- pp. 162-165.
Bessey -- pp. 138-146.

E. SELECTED REFERENCES:

1. FITZPATRICK, H. M. 1930. The lower fungi. Phycomycetes. xi + 331 pp. McGraw-Hill Book Co. New York.
2. GREGORY, C. T. 1912. Spore germination and infection with *Plasmopara viticola*. Phytopaht., 2:235-249.
3. LARGE, E. C. 1940. The advance of the fungi. 488 pp. Henry Holt and Co. New York.
4. MC DONOUGH, E. S. 1937. The nuclear history of *Sclerospora graminicola*. Mycologia, 29:151-173.
5. WESTON, W. H. I. 1924. Nocturnal production of conidia by *Sclerospora graminicola*. Jour. Agr. Res., 27:771-784.

F. GLOSSARY:

1. *Haustorium* (pl. *haustoria*; L. *haustor* = drinker): absorbing organ originating on the hypha of a parasite and penetrating into a cell of the host.
2. *Sterigma* (pl. *sterigmata*; Gr. *sterigma* = support): a small hyphal branch or structure which supports a sporangium, a conidium or a basidiospore.

Class Oömycetes

Order: Peronosporales

Family: Albuginaceae

A. GENERAL REMARKS:

1. The Albuginaceae include but one genus, Albugo, with only a few species. They are all obligately parasitic on flowering plants, causing diseases called "white rusts".

2. The short, club-shaped sporangiophores are borne sub-epidermally and bear more or less spherical sporangia in chains at their tips.

B. PROCEDURE:

Genus: Albugo

3. Examine plant specimens infected with "white rust," and note the white crust on the surface. Scrape off some of this material and mount in lacto-phenol. Note that the crust is composed primarily of sporangia.

4. Study prepared slides showing cross-sections of leaves infected with Albugo. Find the:

   a. somatic hyphae
   b. haustoria
   c. sporangiophores
   d. chains of sporangia
   e. antheridia and oögonia
   f. oöspheres, periplasm
   g. oöspores, thickened wall.

C. QUESTIONS:

1. Characterize the Albuginaceae.
2. What is the relation of the somatic hyphae to the host cells?
3. Of what material is the white, crust-like substance on the surface of the host composed? Explain its origin.
4. Explain the method of formation of sporangia.
5. How do the sporangia of Albugo germinate?
6. How do the Albuginaceae overwinter? Where?
7. What is meant by a systemic infection in a plant host? What does it indicate concerning the overwintering of the fungal parasite?
8. Name as many species of Albugo as you can and give the host for each species.
9. Construct a life cycle diagram for Albugo candida.
10. Compare and contrast the orders of the biflagellate Phycomycetes.

D. TEXT REFERENCES:

Alexopoulos -- pp. 165-170.
Bessey -- pp. 135-138.

E. SELECTED REFERENCES:

1. DAMLE, V. P. 1943. The cytology of Cystopus platensis Speg. and its bearing on the identity and phylogeny of the species. Jour. Indian Bot. Soc., 22:173-185.
2. DAVIS, B. M. 1900. The fertilization of Albugo candida. Bot. Gaz., 29:297-311.
3. ENDO, R. M. and M. B. LIND. 1960. The white-rust of horseradish. Ill. Agr. Exp. Sta., Bull., 655, 56pp.
4. FITZPATRICK, H. M. 1930. The lower fungi. Phycomycetes. xi + 331 pp. McGraw-Hill Book Co. New York.
5. PALM, B. T. 1932. Biological Notes on Albugo. Ann. Mycol., 30:421-426.
6. STEVENS, F. L. 1899. The compound Oosphere of Albugo bliti. Bot. Gaz., 28:149-176, 223-245.
7. STEVENS, F. L. 1901. Gametogenesis and fertilization in Albugo. Bot. Gaz., 32:77-98, 237-261.
8. THIRUMALACHAR, M. J., M. D. WHITEHEAD, and J. S. BOYLE. 1949. Gametogenesis and oöspore formation in Cystopus (Albugo) evolvuli. Bot. Gaz., 110:487-491.
9. VANTERPOOL, T. C. 1959. Oöspore germination in Albugo candida. Can. Jr. Bot., 37:169-172.
10. WILSON, G. W. 1907. Studies in North American Peronosporales I. The genus Albugo. Bull. Torrey Bot. Club, 34:61-84.

Class Plasmodiophoromycetes

A. GENERAL REMARKS:

1. The Plasmodiophoromycetes are predominately obligate endoparasites of aquatic vascular plants, Characeae or aquatic fungi. This group has at times been placed somewhere between the Myxomycetes, Protozoa, and the chytrids. Currently these organism are placed near the biflagellate Oömycetes. The zoöspores are unequally biflagellate with both flagella of the whiplash type and are formed in thin-walled sporangia. The plasmodial thallus lacks any cell wall. Sexual reproduction occurs in some species by fusion of motile amoeboid or biflagellate isogametes, resulting in a zygote.

2. Members of this class invade the host tissues and in many cases cause hypertrophy and hyperplasia of the host and result in tumor-like formations. This class includes only one order, the Plasmodiophorales and one family, the Plasmodiophoraceae.

Order: Plasmodiophorales

Family: Plasmodiophoraceae

A. GENERAL REMARKS:

3. The somatic structure of the Plasmodiophoromycetes is a true plasmodium which develops in very much the same way as that of the Myxomycetes. Other characters which set the Plasmodiophoromycetes as a separate class are: the absence of a fruiting body, the spores being borne singly or in spore balls within the host cells; the cruciform nuclear division; and the presence of an akaryotic stage in the life cycle.

B. PROCEDURE:

Genera: Plasmodiophora, Spongospora

4. Examine cabbage roots infected with *Plasmodiophora brassicae* and compare with normal roots.

5. With a very sharp razor blade cut a very thin section of a diseased rootlet. Mount in water and examine for resting spores.

6. Study a prepared, stained slide of an infected root. Note how the tissues (especially the vascular tissues) are disarranged.

7. Compare infected and normal cells for size and contents. Find some infected cells containing plasmodia and some containing the mature spores of the organism.

8. Examine potato tubers infected with *Spongospora subterranea*.

9. Study prepared slides showing sections through an infected portion of a potato tuber. Note the effect of the parasite on the cells. Find the spore balls of the causal organism.

CLASS PLASMODIOPHOROMYCETES

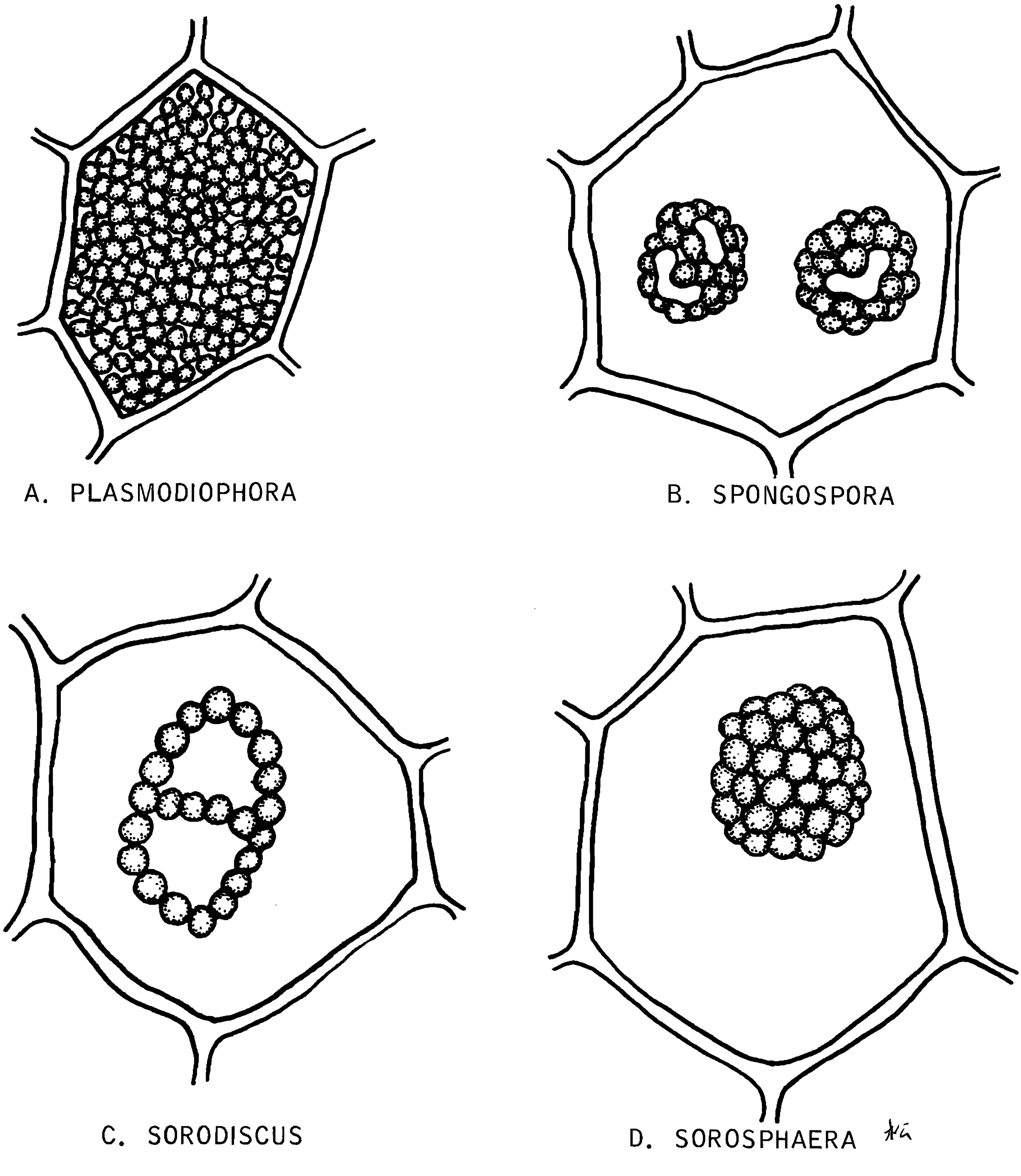


FIG. 30 FOUR TYPES OF SPORE GROUPINGS

Plate 7

10. Compare the distribution of the infected cells in the potato tuber with that of the infected cells in the cabbage root.

Genera: Ligniera, Tetramyxa, Sorodiscus, and Sorosphaera

11. If available, study any of these genera that parasitize aquatic vascular plants, and Chara sp.

Genera: Woronina and Octomyca

12. These two genera are parasitic in the hyphae of some of the aquatic fungi and filaments of Vaucheria sp. If material is available, locate the globose sporangia and the resting spores.

C. QUESTIONS:

1. Construct a probable life cycle diagram for Plasmodiophora brassicae based on the findings of Cook and Schwartz, Tomlinson, and others.
2. Repeat for Spongospora subterranea, on the basis of Karling's discussion, and on the papers of Osborn, Ledingham, and Kole.
3. Briefly explain why the Plasmodiophoromycetes have been relocated so many times in the classification systems.
4. Name the genera of the Plasmodiophoraceae and give the distinguishing characters for each, preferably in the form of a dichotomous key.

D. TEXT REFERENCES:

Alexopoulos -- pp. 176-183.
Bessey -- pp. 31-36; 39; 630-631.
Sparrow -- pp. 768-791.

E. SELECTED REFERENCES:

1. CHUPP, CHARLES. 1917. Studies on clubroot of cruciferous plants. Cornell Agr. Exp. Sta. Bull., 387.
2. COOK, W. R. I. 1933. A monograph of the Plasmodiophorales. Archiv. Protist., 80:174-254.
3. COOK, W. R. I. and E. J. SCHWARTZ. 1930. The life history, cytology, and method of infection of Plasmodiophora brassicae Woron., the cause of finger and toe disease of cabbages and crucifers. Phil. Trans. Royal Soc. London, Ser. B. 218:283-314.
4. ELLIOTT, E. W. 1949. The swarm cells of Myxomycetes. Mycologia. 41:141-171.
5. FITZPATRICK, H. M. 1930. The lower fungi. Phycomycetes. vii + 331 pp. McGraw-Hill Book Co. New York.
6. GOLDIE-SMITH, E. K. 1954. The position of Woronina polycystis in the Plasmodiophoraceae. Amer. Jr. Bot., 41:441-448.
7. GOLDIE-SMITH, E. K. 1956. A new species of Woronina, and Sorodiscus cokeri emended. Jour. Elisha Mitchell Sci. Soc., 72:348-356.
8. KARLING, J. S. 1942. Plasmodiophorales. 144 pp. Published by the author New York.
9. KOLE, A. P. 1954. A contribution to the knowledge of Spongospora subterranea (Wallr.) Lagerh., the cause of powdery scab of potatoes. Tijdschrift over Plantensiekten, 60:1-65.
10. KOLE, A. P. and A. J. GIELINK. 1961. Electron microscope observations on the flagella of the zoösporangial zoöspores of Plasmodiophora brassicae and Spongospora subterranea. Kininkl. Nederl. Akad. Wetensch. Proc. ser. C., 64:157-161.
11. KOLE, A. P. and A. J. GIELINK. 1962. Electron microscope observations on the zoöspores arising from the resting spores of Plasmodiophora brassicae Koninkl. Nederl. Akad. Wetensch. Proc. ser. C., 65:117-121.
12. LEDINGHAM, G. A. 1934. Zoöspore ciliation in the Plasmodiophorales. Nature, 133:534.
13. LEDINGHAM, G. A. 1935. Occurrence of zoösporangia in Spongospora subterranea (Wallroth) Lagerheim. Nature, 135:394-395.
14. OSBORN, T. G. B. 1911. Spongospora subterranea (Wallroth) Johnson Ann. Bot., 25:327-341.
15. PALM, B. T. and MYRLE BURK. 1933. The taxonomy of the Plasmodiophoraceae. Archiv Protist., 79:263-276.
16. SPARROW, F. K., JR. 1960. Aquatic Phycomycetes. xxv + 1187 pp. The University of Michigan Press, Ann Arbor.
17. TOMLINSON, J. A. 1958. Crook root of water cress. III. The causal organism Spongospora subterranea (Wallr.) Lagerh. f. sp. nov. Trans. Brit. Mycol. Soc., 41:491-498.

18. WEBB, P. C. R. 1949. Zoosporangia believed to be those of Plasmodiophora brassicae in the root hairs of non-cruciferous plants. Nature, 163:608.
19. WORONIN, M. 1934. Plasmodiphora brassicae the cause of cabbage hernia. (English translation. Phytophathological Classics No. 4. 32 pp.)

F. GLOSSARY:

1. Akaryotic (Gr. a = not + karyon = nut, nucleus): a phase in the life cycle of the Plasmodiophorales during which the nucleoplasm loses its affinity for stains.
2. Cruciform division (L. crux = cross + forma = image): a type of nuclear division during which the nucleolus divides and persists in the daughter nuclei. At a certain stage, the division figure has the appearance of a cross. Cruciform division is characteristic of the Plasmodiophoraceae.
3. Endoparasite (Gr. endos = inside + parasitos = parasite): a parasite completing most of its life cycle inside a host.
4. Hyperplasia (Gr. hyper = over + plasis = molding, formation): excessive multiplication of cells; abnormal rate of cell division.
5. Hypertrophy (Gr. hyper = over + trophe = food): excessive enlargement of cells.

Class Zygomycetes

A. GENERAL REMARKS:

1. This class of fungi has well-developed mycelium, with septa developed in some of the older hyphae. The vigorously growing hyphae are normally non-septate. Asexual reproduction is accomplished by means of non-motile sporangiospores (aplanospores). Such spores lack flagella and are usually air borne. Sexual reproduction takes place by the fusion of two gametangia, which are similar in structure but which may or may not be similar in size, resulting in the formation of a thick-walled zygospore.

2. The Zygomycetes are subdivided into three orders: the Mucorales, the Entomophthorales, and the Zoöpagales.

Order: Mucorales

A. GENERAL REMARKS:

3. The Mucorales have coenocytic, well-developed richly branched mycelium which may become septate in older cultures. Most members are usually terrestrial saprobes on plant or animal tissues. Asexually, spores are usually formed within a sporangium or a sporangiole. Under certain conditions the hyphae may fragment or form thick-walled intercallary chlamydospores. Sexual reproduction takes place by gametangial copulation (fusion of two more or less similar gametangia), and results in the formation of a thick-walled zygospore.

4. The asexual reproductive structures in this order range from a multispored sporangium, through a few-spored sporangiole, to a monosporous sporangiole which approaches the structure of a conidium, practically attaining that structure in the genus Cunninghamella.

5. A few species are pathogenic on plants while several other species have been associated with animal and human diseases. A number of species are of considerable importance in the decay of fruits and vegetables. Species of Rhizopus and Mucor are important in the saccharification of starch, and the former genus is also used in lactic acid fermentation.

6. The order Mucorales is subdivided into eleven families: Mucoraceae, Thamnidiaceae, Cunninghamellaceae, Choanephoraceae, Pilobolaceae, Mortierellaceae, Endogonaceae, Syncephalastraceae, Piptocephalidaceae, Dimargaritaceae, and Kickxellaceae. Not all of the families will be considered here.

Family: Mucoraceae

Genera: Rhizopus, Absidia, Mucor, Phycomyces, Zygorhynchus

A. GENERAL REMARKS:

7. The sporangia of this family have columellae, and the walls have no cutin. The zygospores lack any interwoven hyphae over the surface of their thick-walls, but appendages may be present.

B. PROCEDURE:

8. Moisten a piece of bread, place in deep dish or Petri dish, and inoculate with Rhizopus stolonifer (R. nigricans). In addition, inoculate a Petri dish containing cornmeal agar or other media with R. stolonifer, and maintain both cultures at room temperature for three days. Inoculate plus and minus strains of R. stolonifer, one strain near one side of the Petri dish and the other strain near the other side. Inoculate a homothallic species such as Zygorhynchus sp. in the center of another Petri dish containing cornmeal agar. Maintain at room temperature for 3 to 5 days.

9. Examine the piece of bread under low magnification to locate the cottony growth consisting of hyphae, stolons, rhizoids, sporangiophores, and sporangia.

10. Place the Petri dish containing R. stononifer on the stage of the microscope or the stereomicroscope, and observe the structures listed in paragraph 9 through the glass.

11. Mount some of the cottony mass from the Petri dish culture on a slide. Locate sporangia filled with spores, broken sporangia with the columellae visible on the top of the sporangiophores, and the other structures of the fungus.

12. Study prepared slides showing various stages in zygospore formation. Locate stages which show progametangia, gametangia, zygotes, suspensors, and zygospores.

13. Observe Petri dishes containing plus (+) and minus (-) strains. Note the distinct line of zygospores which have been formed at the line of contact between the two strains. Compare the location of the zygospores of Rhizopus nigricans, a heterothallic species, with that in any homothallic species available.

14. Examine cultures of other genera in this family, such as Absidia, Mucor, Phycomyces, and Zygorhynchus. Familiarize yourself with the characteristics of each and compare them with Rhizopus stolonifer.

Family: Thamnidiaceae

Genus: Helicostylum

A. GENERAL REMARKS:

15. Large sporangia and sporangioles are usually formed on the same sporangiophore by members of this family.

B. PROCEDURE:

16. Examine a culture of any species of Helicostylum, under the dissecting microscope. Locate the large sporangia terminating some hyphae and the whorls of sporangioles on circinate lateral branches.

17. Mount a portion of the colony on a slide and observe the structure of the sporangia, sporangioles, and spores. Note that columellae are present in the sporangia only. Some hyphae may terminate in a spine.

18. The zygospores are similar to those in the Mucoraceae.

Family: Cunninghamellaceae

Genus: Cunninghamella

A. GENERAL REMARKS:

19. The genera in this family reproduce asexually by conidia only. Zygospores are formed as in Mucor.

B. PROCEDURE:

20. Examine a culture of a species of Cunninghamella. Note the ramose conidiophores with a vesicular swelling at the tip of each branch where conidia are borne.

21. If heterothallic strains are available, cross the two and note that the zygospores are borne in a manner similar to Mucor.

Family: Choanephoraceae

Genera: Blakeslea, Choanephora

A. GENERAL REMARKS:

22. These genera always possess sporangia and either sporangiola or conidia in addition.

23. Study a culture of a species of Choanephora. Observe the large sporangia with columellae and the numerous small conidia. Under high magnification look for striations in the spore walls of the conidia.

24. Study a culture of Blakeslea trispora. Observe a large sporangium with a columella and the numerous small sporangioles on the vesicular tip of the sporangiophore. Note the hair-like appendages and the striations on the spores.

25. If plus and minus strains are available study the development of the zygospores.

Family: Pilobolaceae

Genus: Pilobolus

A. GENERAL REMARKS:

26. Members of this family have a heavily cutinized sporangial wall and a columella. The entire sporangium is discharged violently at maturity from the sporangiphore and adheres to any solid object it strikes. The zygospores are not surrounded by any interwoven hyphae.

B. PROCEDURE:

27. Place some fresh horse manure in a moist glass chamber and maintain at room temperature for 3 or 4 days. Place some moistened horse manure in an open glass container, insert in cardboard box. Punch a small hole in the center of the box top or side. After 3 or 4 days open the box and observe the accuracy of the "cap throwers" (Pilobolus sp.) in striking the small hole. Note the phototropic response of the sporangiophores.

28. Mount a sporangiophore with the enlarged basal portion, in a drop of water and study under low power. Observe the sporangium with the thick upper wall, the columella, the sporangiospores, and the sub-sporangial vesicle. The basal portion, which is frequently swollen, is known as the trophocyst.

29. If material is available study the zygospore with the tong-like suspensors.

Family: Mortierellaceae

Genus: Mortierella

A. GENERAL REMARKS

30. This family is distinguished by the lack of a columella in the sporangium. The zygospores are enclosed within an envelop of interwoven hyphae.

B. PROCEDURE:

31. Observe the procumbent habit of the mycelium in a culture of Mortierella sp. Compare this habit of growth with that of the Mucoraceae.

32. After mounting a portion of the colony of Mortierella, observe that the branched or unbranched sporangiophores taper toward the tip where sporangia without columellae are attached. Look for the spiny stylospores (conidia) at the tips of short hyphal branches.

33. Study successive stages of zygospore development and note that mature zygospores are surrounded by a hyphal envelop.

34. If other genera are available, compare them with Mortierella.

Family: Syncephalastraceae

Genus: Syncephalastrum

A. GENERAL REMARKS:

35. This family has branched sporangiophores, and modified sporangia (merosporangia) that are cylindrical or elongate with few spores in each. Zygospores have not been observed.

B. PROCEDURE:

36. Study a species of Syncephalastrum. Note the branched sporangiophores with globose heads bearing rod-shaped sporangia on sterigmata. Note the arrangement of the spores after the sporangial walls have disappeared. No sexual reproduction is known.

Family: Piptocephalidaceae

Genera: Dispira, Piptocephalis, Syncephalis

A. GENERAL REMARKS:

37. Many of the genera in this family parasitize other members of the Mucorales. The sporangiophores are unbranched or dichotomously branched with cylindrical merosporangia. Zygospores are formed between tong-like suspensors.

B. PROCEDURE:

38. Observe the dichotomously branched sporangiophores with deciduous cylindrical merosporangia at the apex of each branch in Piptocephalis. Compare with other genera.

39. Locate zygospores with tong-like suspensors. Compare with zygospores in other families.

C. QUESTIONS:

1. What are the characteristics of the Mucorales?
2. How do the Mucorales differ from other fungi you have studied so far in their method of sexual reproduction?
3. What is meant by plus and minus strains?
4. Explain homothallism and heterothallism.
5. What is the economic importance of the Mucorales?
6. Construct a probable life cycle diagram for Rhizopus stolonifer.
7. Characterize the families of the Mucorales.
8. Explain the mechanism of sporangial discharge in Pilobolus.
9. Discuss the nuclear behavior of the Mucorales during sexual reproduction. How does the compatibility factor segregate as indicated by the behavior of the mycelia produced by spores from the zygosporangia?
10. Describe the probable evolution of the sporangium and the origin of the conidium, as exemplified by present day forms of the Mucorales.
11. What are merosporangia? Sporocladia?

D. TEXT REFERENCES:

Alexopoulos -- pp. 184-201.
Bessey -- pp. 150-172.

E. SELECTED REFERENCES:

1. AL-DOORY, YOUSEF. 1959. The effect of various substances on the oxygen uptake of Rhizopus oryzae. Mycologia 51:851-854.
2. BENJAMIN, C. R., and C. W. HESSELTINE. 1959. Studies on the genus Phycomyces. Mycologia 51:751-771.
3. BENJAMIN, R. K. 1958. Sexuality in the Kickxellaceae. El Aliso, 4:149-169.
4. BENJAMIN, R. K. 1959. The merosporangiferous Mucorales. El Aliso, 4:321-453.
5. BLAKESLEE, A. F. 1904. Sexual reproduction in the Mucorineae. Proc. Amer. Acad. Arts and Sci., 40:205-319.
6. BLAKESLEE, A. F. 1913. Conjugation in the heterogamic genus Zygorhynchus. Mycol. Centralbl., 2:241-244.
7. BULLER, A. H. R. 1934. Researches of fungi. Vol. 6. 224 pp. Longmans, Green and Co., London.
8. CALLON, E. C. 1940. The morphology, cytology, and sexuality of the homothallic Rhizopus sexualis (Smith) Callon. Ann. Bot., n.s., 4:791-818.
9. CHRISTENBERRY, G. A. 1940. A taxonomic study of the Mucorales in the southeastern states. Jour. Elisha Mitchell Sci. Soc., 56:333-366.
10. CUTTER, V. M., JR. 1942. Nuclear behavior in the Mucorales. I. The Mucor pattern. Bull. Torrey Bot. Club, 69:480-508. II. The Rhizopus, Phycomyces, and Sporodinia patterns. Bull. Torrey Bot. Club, 69:592-616.
11. CUTTER, V. M., JR. 1946. The genus Cunninghamella. (Mucorales.) Farlowia, 2:321-343.
12. FITZPATRICK, H. M. 1930. The lower fungi. Phycomycetes. McGraw-Hill Book Co., New York.
13. GAUGER, WIL. 1961. The germination of zygospores in Rhizopus stolonifer. Am. Jr. Bot., 48:427-429.
14. HESSELTINE, C. W. 1955. Genera of Mucorales with notes on their synonymy. Mycologia, 47:344-363.
15. HESSELTINE, C. W., and R. F. ANDERSON. 1957. Microbiological production of carotenoids. L. Zygospores and carotene produced by intraspecific and interspecific crosses of Choanephoraceae in liquid media. Mycologia, 49:449-452.
16. HESSELTINE, C. W., and C. R. BENJAMIN. 1957. Notes on the Choanephoraceae. Mycologia, 49:723-733.
17. HESSELTINE, W. C., C. R. BENJAMIN, and B. S. MEHROTRA. 1959. The genus Zygorhynchus. Mycologia, 51:173-194.
18. KEENE, MARY L. 1919. Studies of the zygospore formation in Phycomyces nitens. Trans. Wis. Acad. Sci., 19:1195-1220.
19. PAGE, R. M. 1960 (1961). The effect of ammonia on growth and reproduction of Pilobolus kleinii. Mycologia, 52:480-489.
20. POITRAS, A. W. 1955. Observations on asexual and sexual reproductive structures of the Choanephoraceae. 1955. Mycologia, 47:702-713.

21. POVAH, A. H. W. 1917. A critical study of certain species of Mucor. Bull. Torrey Bot. Club, 44:241-259.
22. SWINGLE, D. B. 1903. Formation of spores in the sporangia of Rhizopus nigricans and of Phycomyces nitens. U. S. Dept. Agr. Plant. Ind. Bull., 37:1-40.

F. GLOSSARY:

1. Aplanospore (Gr. a = not + planetes = wanderer + sporos = seed, spore): a non-motile spore.
2. Chlamydospore (Gr. chlamys = coat + sporos = seed, spore): a hyphal cell, enveloped by a thick cell wall, which eventually becomes separated from the parent hypha and behaves as a resting spore.
3. Conidiophore (Gr. konis = dust + phoreus = bearer): a specialized hypha bearing conidia.
4. Merosporangium (pl. merosporangia; Gr. meros = portion + sporangium): a cylindrical sporangiolum.
5. Progametangium (pl. progametangia; Gr. pro = before + gametangium): a cell which gives rise to a gametangium.
6. Sporangiole (sporangium + -olum = Latin dimin. suffix): a small sporangium containing a few spores, or only a single spore.
7. Sporocladium (pl. sporocladia; Gr. sporos = spore - clados = branch): a special type of fertile branch of a sporangiophore which bears merosporangia.
8. Suspensor (L. suspensare = to suspend): one of a pair of cells between which a zygospore is suspended.
9. Stolon (Fr. stolon = a runner): a hypha of the Mucorales which connects two groups of rhizoids.
10. Trophocyst (Gr. trophe = food + kystis = bladder): a swollen cell basal to the sporangiophore of Pilobolus.

Class Zygomycetes

Order: Entomophthorales

A. GENERAL REMARKS:

1. Most species of Entomophthorales are parasitic on plant lice, flies, scale insects, and larvae of butterflies or beetles; some, however, are saprobic, living on lizard or frog dung, or on other non-living organic matter.

2. The mycelium is less extensively branched when compared with the Mucorales. There is a definite tendency to form septa and then fragment into hyphal bodies. These may multiply by division or budding and eventually produce conidiophores or resting spores. The conidia are forcibly discharged in most species. However in the genus Basidiobolus, the sporangia (or "conidia") contain sporangiospores. Thick-walled resting spores are formed by most species, developing either by gametangial copulation as zygospores or parthenogenetically as azygospores.

B. PROCEDURE:

Family: Entomophthoraceae

Genus: Entomophthora

3. Examine a species of Entomophthora growing in insects or in culture. If available, examine the halo-like zone of conidia around a dead house fly on a windowpane. This is due to the fly fungus, Entomophthora muscae. Study prepared slides showing sections of house flies infected by this fungus. Note the conidiophores protruding out between the segments of the abdomen, and the small hyphal bodies that become distributed throughout the body of the host, replacing most of the tissues within the integument of a fly.

4. If zygospores are present, note the thick wall and similarity to the oöspores of some members of the Peronosporales.

Family: Basidiobolaceae

Genus: Basidiobolus

5. Obtain frog or snake dung and place it on water agar or acid malt agar. Watch for sporangiophore development of Basidiobolus sp. within 24 hours. The sporangia that form are shot off by force toward a light source. If possible observe the discharge of the sporangia. These later form sporangiospores.

6. Observe the developmental stages of zygospore formation.

7. Study a culture of a species of Conidiobolus producing conidiophores and conidia. Observe the conidia being discharged. Look for the various stages of zygospore development in the agar.

C. QUESTIONS:

1. Why are the Entomophthorales classified as Zygomycetes?
2. Are the asexual spores true conidia? Discuss.
3. How are the conidia discharged? How do they germinate?
4. Diagram the life cycle of Entomophthora muscae.
5. Are these fungi important economically?
6. Compare the zygospore formation in the Entomophthorales with that of the Mucorales.

D. TEXT REFERENCES:

Alexopoulos -- pp. 201-205.
Bessey -- pp. 172-177.

E. SELECTED REFERENCES:

1. COUCH, J. N. 1939. A new Conidiobolus with sexual reproduction. Am. Jr. Bot., 26:119-130.
2. DRECHSLER, C. 1956. Supplementary developmental stages of Basidiobolus ranarum and Basidiobolus haptosporus. Mycologia, 48:655-676.
3. DRECHSLER, C. 1961. Two species of Conidiobolus often forming zygospores adjacent to antheridium-like distentions. Mycologia, 53:278-303.
4. EMMONS, C. W., and C. H. BRIDGES. 1961. Entomophthora coronata, the etiologic of a phycomycosis of horses. Mycologia, 53:307-312.
5. FITZPATRICK, H. M. 1930. The lower fungi. Phycomycetes. McGraw-Hill Book Co., Inc. New York.
6. GOLDSTEIN, BESSIE. 1923. Resting spores of Empusa muscae. Bull. Torrey Bot. Club, 50:317-328.
7. GOLDSTEIN, BESSIE. 1927. An Empusa disease of Drosophila. Mycologia, 19:97-109.
8. OLIVE, E. Q. 1906. Cytological studies on the Entomophthoreae. Bot. Gaz., 41:192-208, 229-261.
9. SAWYER, W. H. 1929. Observations on some entomogenous members of the Entomophthoraceae in artificial culture. Am. Jr. Bot., 16:87-121.
10. THAXTER, R. 1888. The Entomophthoreae of the United States. Mem. Boston Soc. Nat. Hist., 4:133-201.

F. GLOSSARY:

1. Azygospore (Gr. a = not + zygos = yoke + sporos = spore): a zygospore formed parthenogenetically without gametangial fusion.
2. Hyphal body (Gr. Hyphe = web): a fragment of the mycelium of the Entomophthorales.
3. Parthenogenesis (Gr. parthenos = virgin - genesis = birth): the development of the normal product of sexual reproduction without fertilization.

Class Zygomycetes

Order: Zoöpagales

A. GENERAL REMARKS:

1. Most species of the Zoöpagales are parasitic on soil-inhabiting and aquatic animals: amoebae, nematodes, and insect larvae. The hyphae are nonseptate at first; later some cross walls may form. Asexual reproduction is by the formation of conidia laterally or apically on branches. The conidia may be in chains. The zygospores are spherical to boat-shaped.

B. PROCEDURE:

Family: Zoöpagaceae

Genera: Endocochlus, Cochlonema, Zoöpage and Cystopage

2. The fungi in this family produce various types of haustoria or internal mycelium within the host amoebae and nematodes, and long slender external hyphae that give rise to conidia.

3. Examine parasitized amoebae, nematodes or insect larvae containing representative genera in this family. Locate the internal mycelium, haustoria, if present, conidia and zygospores if present.

C. QUESTIONS:

1. Characterize the order Zoöpagales.
2. How does this order differ from the Entomophthorales?

D. TEXT REFERENCES:

Alexopoulos -- pp. 205-207.
Bessey -- pp. 177-180.

E. SELECTED REFERENCES:

1. DRECHSLER, C. 1935. Some conidial phycomycete destructive to terricolous amoebae. Mycologia, 27:176-205.
2. DRECHSLER, C. 1957. A nematode-capturing phycomycete forming chlamydospores terminally on lateral branches. Mycologia, 49:387-391.
3. DRECHSLER, C. 1959. Several Zoöpagaceae subsisting on a nematode and on terricolous amoebae. Mycologia, 51:787-823.
4. DUDDINGTON, C. L. 1956. The predacious fungi: Zoöpagales and Moniliales. Biol. Rev., 31:152-193.

Class Trichomycetes

A. GENERAL REMARKS:

1. The Trichomycetes are a group of fungi which grow attached to the intestinal tract or the exoskeleton of arthropods. The coenocytic hyphae are not as well-developed as those of the Zygomycetes. Asexual reproduction is by means of sporangiospores or conidia.

2. The Trichomycetes are divided into a number of orders. One order, the Eccrinales will be considered.

Order: Eccrinales

A. GENERAL REMARKS:

3. Representatives of this order have a simple, unbranched thallus with a holdfast. Sporangiospores are of two types, uninucleate or multinucleate. The uninucleate spores become thick-walled in some species. Little is known about sexual reproduction in this order.

B. PROCEDURE:

Family: Eccrinaceae

Genus: Enterobryus

4. Locate the holdfast at the proximal end of the chitinous wall in the gut. Observe the unbranched coenocytic thallus or hypha.

5. Find the sporangia with the sporangiospores at the hyphal tip.

6. If other genera are available such as Palavascia, compare the differences in structures and appearance.

C. QUESTIONS:

1. On what bases are the Trichomycetes separated from the Zygomycetes?
2. How many orders are there in this Class? How are they separated?

D. TEXT REFERENCES:

Alexopoulos -- pp. 211-214.
Bessey -- pp. 180-183.

E. SELECTED REFERENCES:

1. LICHTWARDT, R. W. 1958. An Enterobryus from the milliped Boraria carolina (Chamberline). Mycologia, 50:55-561.
2. LICHTWARDT, R. W. 1960 (1961). Taxonomic position of the Eccrinales and related fungi. Mycologia, 52:410-428.
3. MANIER, JEAHANNE-FRANCOISE. 1955. Classification et nomenclature des Trichomycètes. Ann. Sci. Nat. Zoo., Ser. 11, 17:395-397.
4. SÖRGEL, G. 1953. Zur Systematik der Trichomyceten. Arch. für Mikrobiol., 18:391-396.
5. TUZET, ODETTE, and JEHANNE-FRANCOISE MANIER. 1954. Importance des cultures de Trichomycètes pour l'étude du cycle et de la classification de ces organismes. Compt. Rend., 238:1904-1905.

CLASS ASCOMYCETES

SOMATIC STRUCTURES

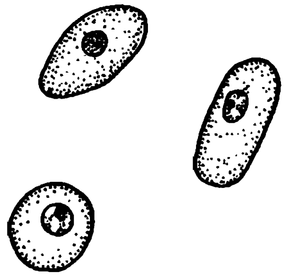


FIG. 31 SINGLE CELLS

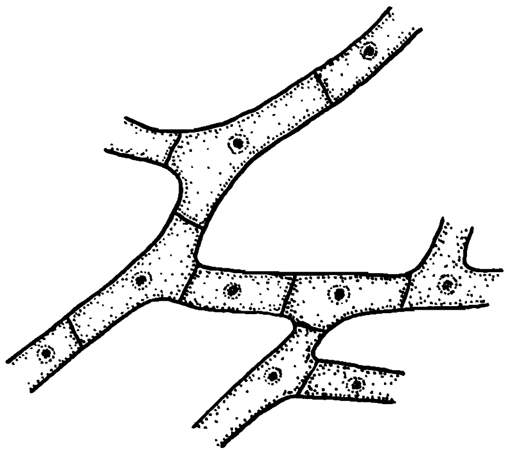


FIG. 32 HYPHAE

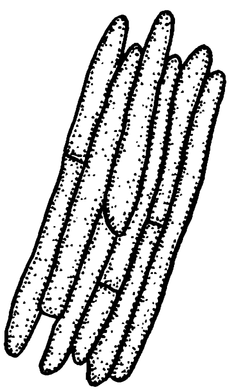


FIG. 33 PROSENCHYMA

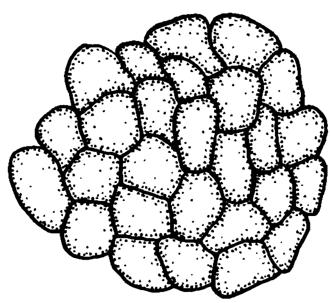


FIG. 34 PSEUDOPARENCHYMA

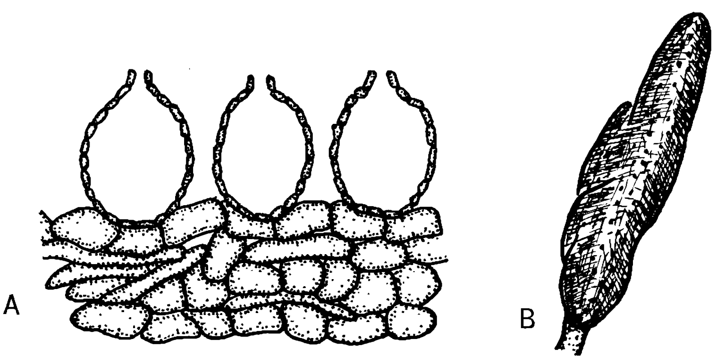


FIG. 35 STROMATA

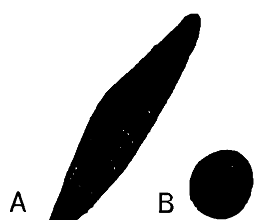


FIG. 36 SCLEROTIA

Plate 8

CLASS ASCOMYCETES

ASEXUAL REPRODUCTION

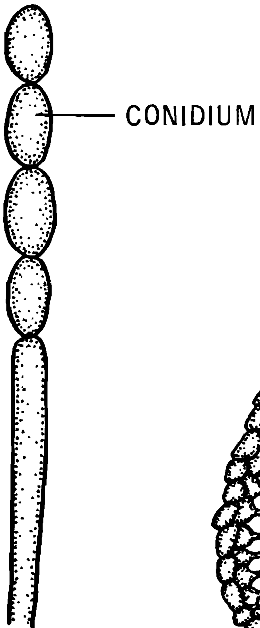


FIG. 37
SIMPLE CONIDIOPHORE

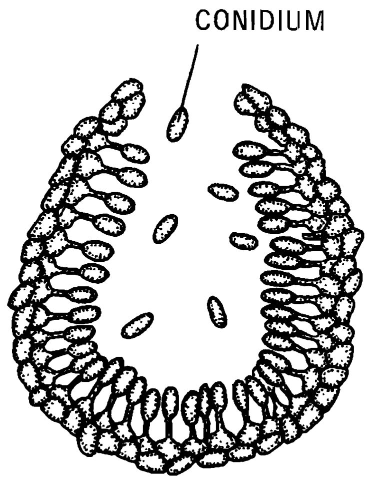


FIG. 38 PYCNIDIUM

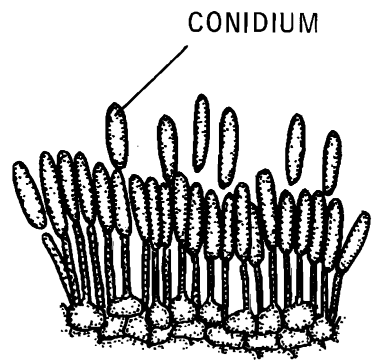


FIG. 39 ACERVULUS

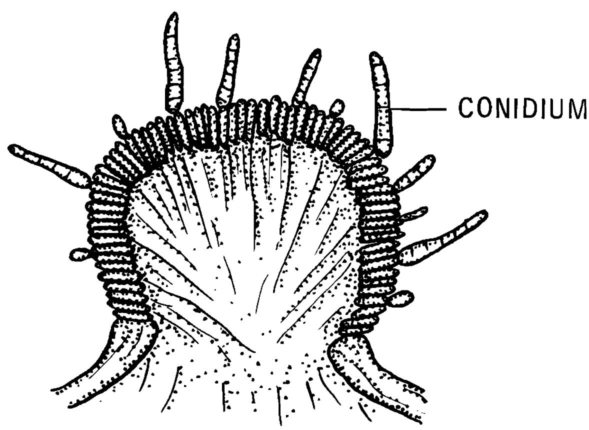


FIG. 40 SPORODOCHIUM

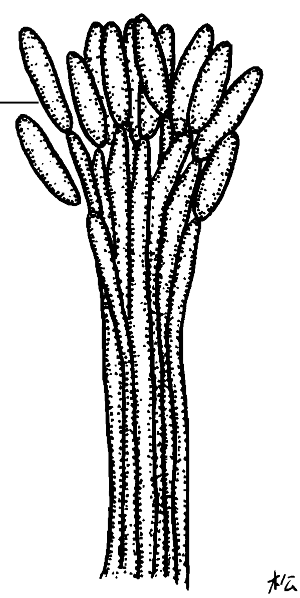


FIG. 41 SYNNEMA

Plate 9

CLASS ASCOMYCETES

SEXUAL REPRODUCTION

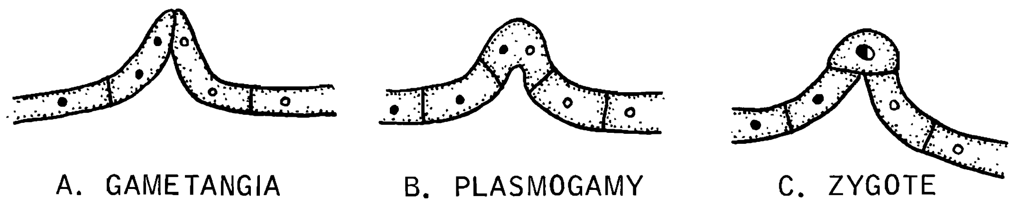


FIG. 42 GAMETANGIAL COPULATION

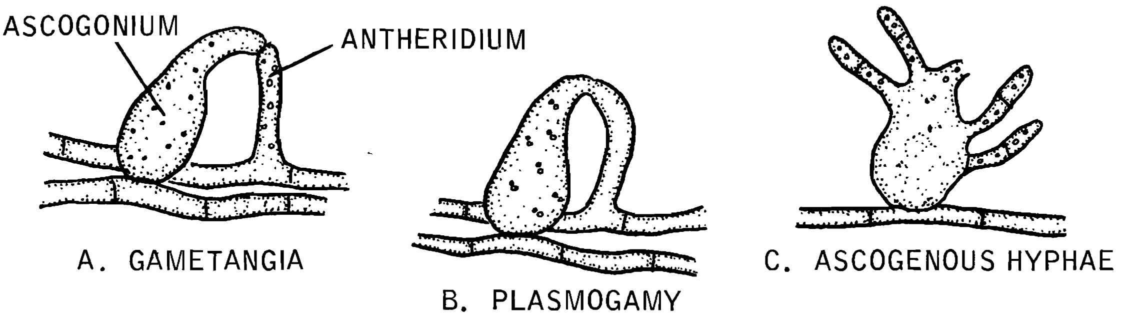


FIG. 43 GAMETANGIAL CONTACT

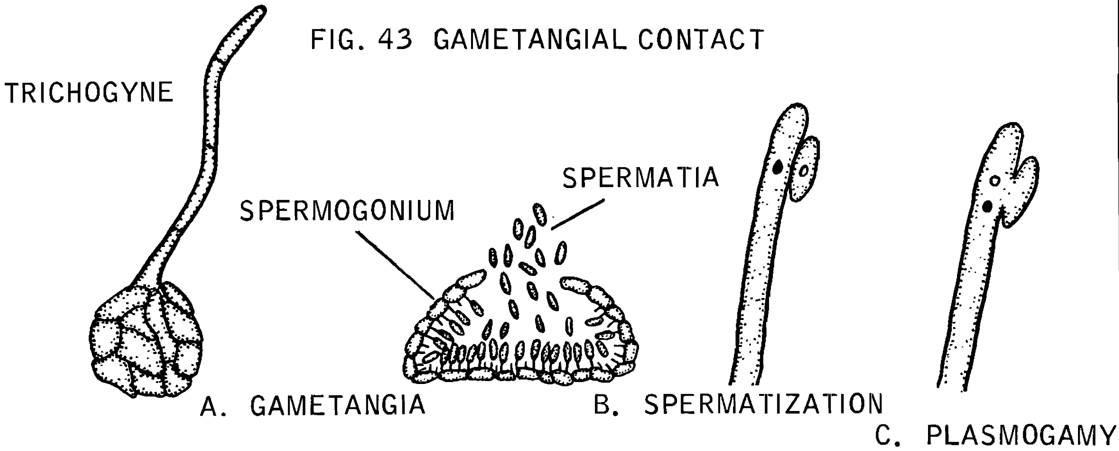


FIG. 44 SPERMATIZATION

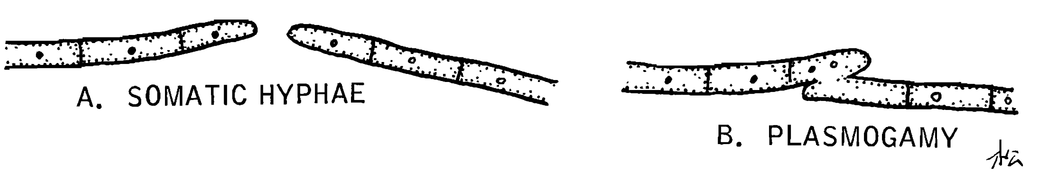


FIG. 45 SOMATOGAMY

Plate 10

CLASS ASCOMYCETES

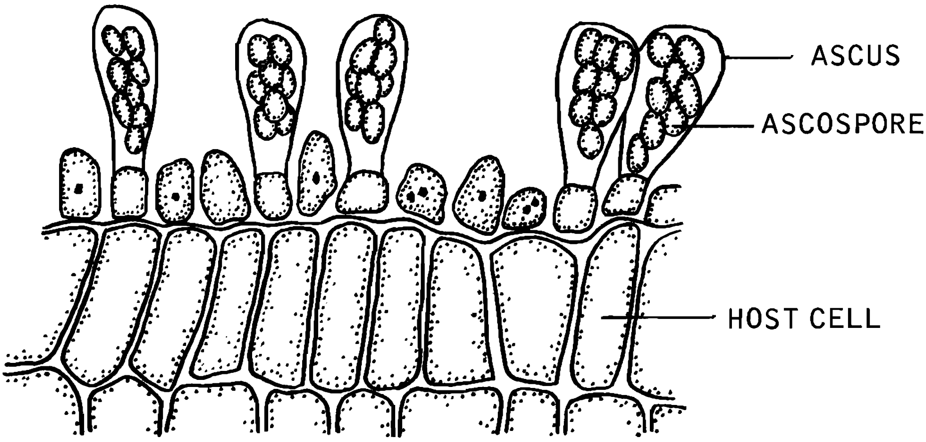


FIG. 46 NAKED ASCI ON THE SURFACE OF THE HOST

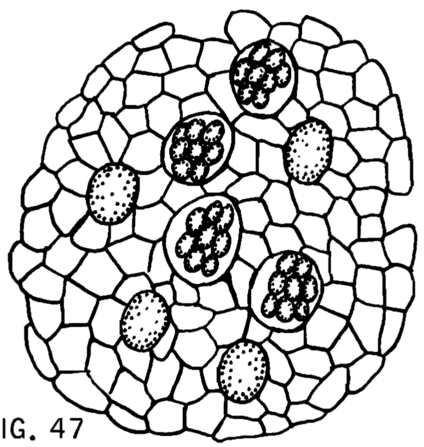


FIG. 47
SECTION THROUGH A CLEISTOTHECIUM WITH SCATTERED ASCI

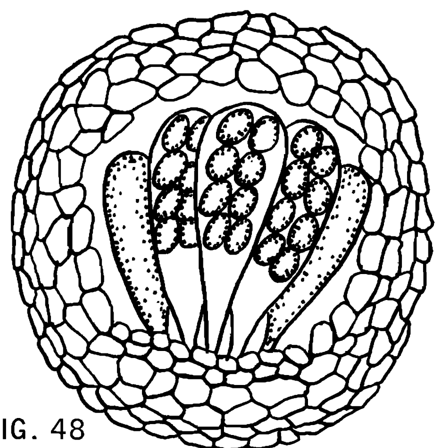


FIG. 48
SECTION THROUGH A CLEISTOTHECIUM WITH BASAL ASCI

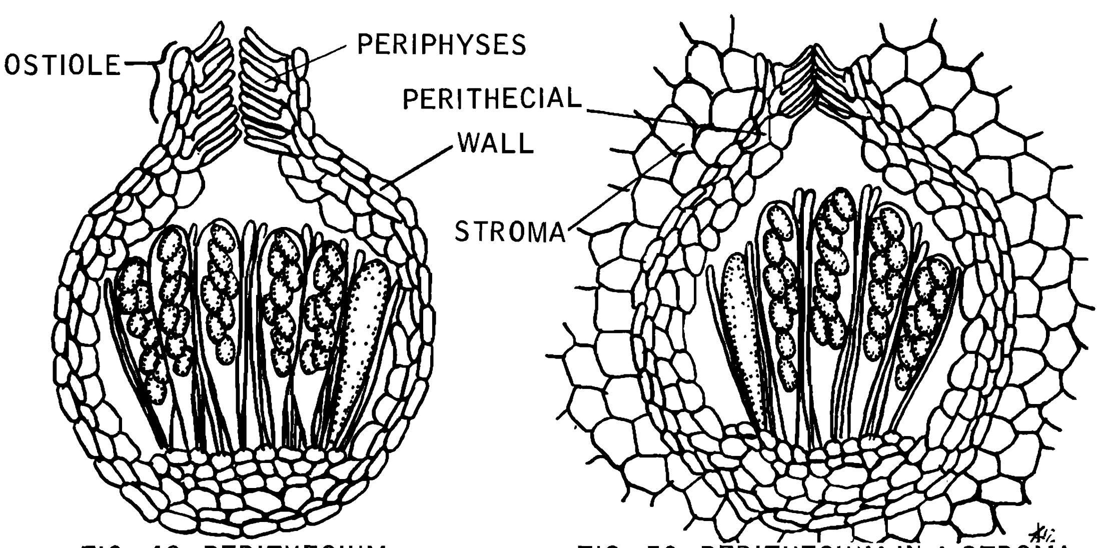


FIG. 49 PERITHECIUM

FIG. 50 PERITHECIUM IN A STROMA

Plate 11

CLASS ASCOMYCETES

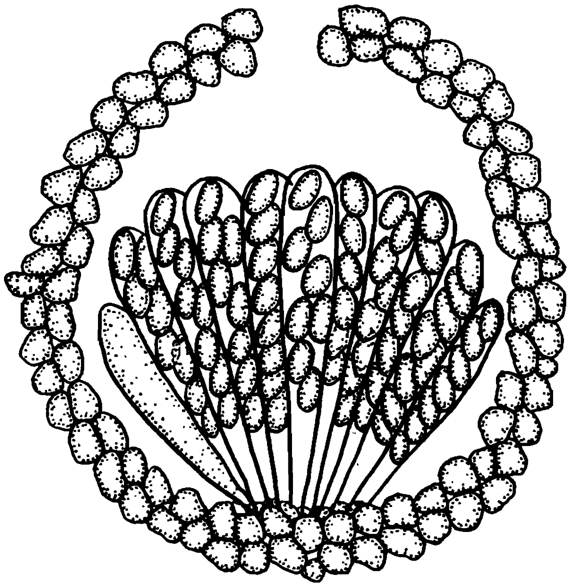


FIG. 51 PSEUDOTHECIUM

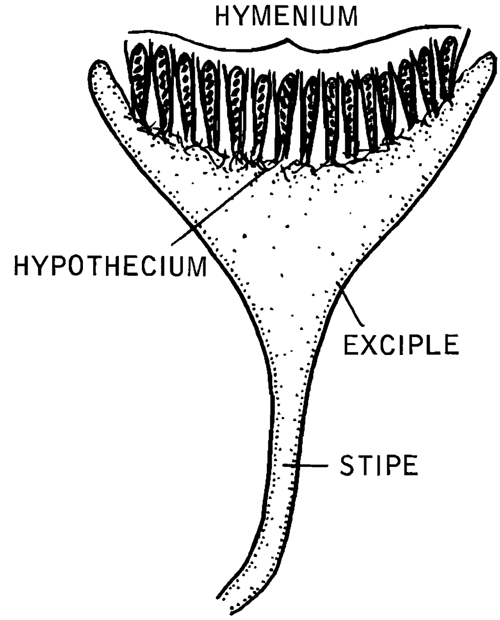


FIG. 52 APOTHECIUM

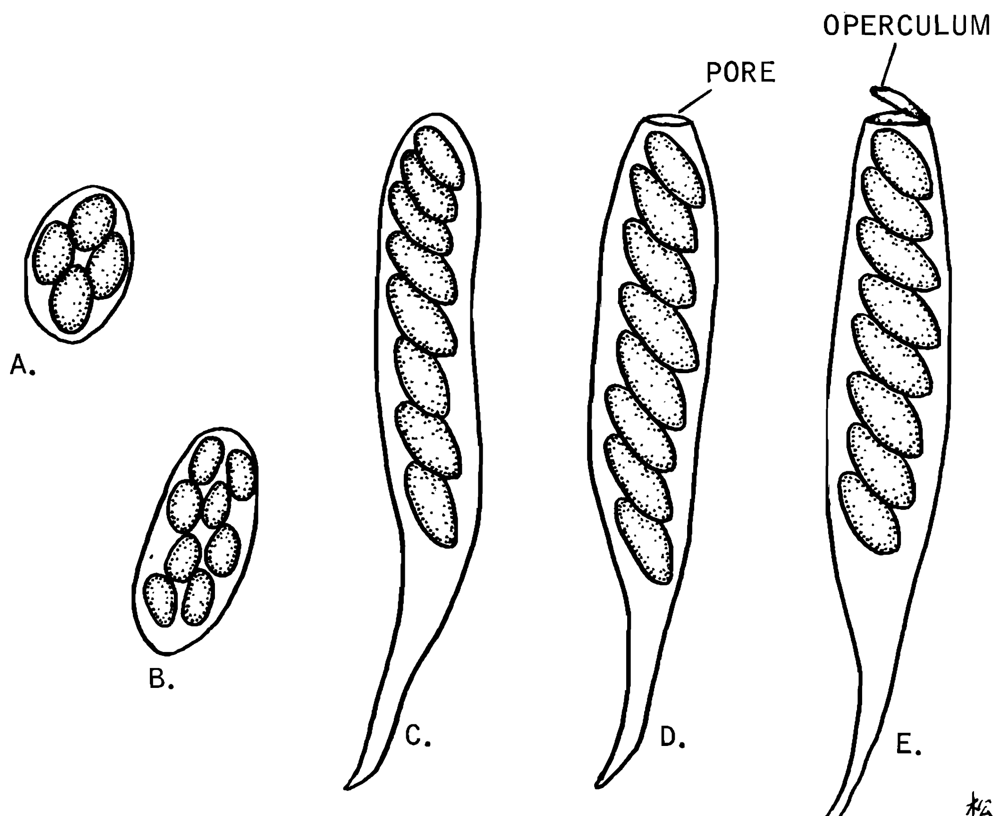


FIG. 53 VARIOUS TYPES OF ASCI

Plate 12

Class Ascomycetes – Sub-Class Hemiascomycetidae

A. GENERAL REMARKS:

1. The Ascomycetes are fungi with septate mycelium which produce spores in sac-like structures called asci (sing. ascus). Karyogamy and meiosis occur in the young ascus and eight haploid uninucleate ascospores are typically formed by a process known as free cell formation.

2. Three sub-classes of Ascomycetes are recognized: the Hemiascomycetidae, the Euascomycetidae, and the Loculoascomycetidae. In the Hemiascomycetidae the asci are naked without any ascocarp being formed. They are developed directly from a zygote cell; no ascogenous hyphae are formed.

3. The Hemiascomycetidae are divided into two orders: the Endomycetales and the Taphrinales. In the former, the asci arise directly from the fusion of two cells; in the latter, from chlamydospore-like binucleate ascus mother cells.

Order: Endomycetales

A. GENERAL REMARKS:

4. The Endomycetales include the yeasts and various other yeast-like organisms. They are all saprobic, living on materials rich in sugars, such as the nectar of flowers, the surface of fruits, etc. Four families are generally recognized: Ascoideaceae, Endomycetaceae, Saccharomycetaeae, and Spermophthoraceae.

B. PROCEDURE:

Family: Ascoideaceae
Genus: Dipodascus

5. In the Ascoideaceae the asci contain a large, indefinite number of ascospores.

6. Study a culture of Dipodascus uninucleatus or other species. Note the characteristic growth. Mount a portion of the mycelium, and observe the septa and the more or less irregular cells.

7. Study ascus formation by searching your slide for as many stages in ascus development as you can find.

8. Note the large number of ascospores in the mature asci, and the method by which ascospores escape.

Family: Endomycetaceae

Genera: Eremascus, Endomyces, Endomycopsis

9. The Endomycetaceae differ from the Ascoideaceae in that their asci contain eight or fewer ascospores and in that the gametangia are generally uninucleate. They include the yeast-like fungi which form mycelium.

10. Study Eremascus fertilis in the same way you studied Dipodascus. Note the similarities and differences between the two organisms.

11. Study Endomyces and Endomycopsis. Note the large oidia in the former and the small, numerous buds in the latter. Search for asci and note the number of spores and the variation in size and shape.

Family: Saccharomycetaceae

Genera: Schizosaccharomyces, Saccharomyces, Hansenula, Nematospora

12. The Saccharomycetaceae are the true yeasts. Mycelium is typically scanty or lacking. False mycelium may be formed by budding.

13. Study various genera of true yeasts as follows:

    a. Schizosaccharomyces octosporus. This is a "fission" yeast. Mount a small portion of the colony and observe the somatic cells. Find some which are undergoing division. Study the formation of asci and ascospores by searching your slide for various stages of development beginning with the copulation of two cells and ending with the mature ascus. Note the variation in the number of ascospores in the asci.

    b. Saccharomyces cerevisiae. This is a budding yeast. Compare and contrast with Schizosaccharomyces octosporus.

    c. Hansenula saturnus. Study the asci and the peculiar ascospores which have a ridge around their middle resembling the ring of the planet Saturn (hence the specific epithet). Other species of Hansenula have different ascospores.

14. Sexual agglutination procedure for yeasts (Wickerham, 1958). Using two strains of Saccharomyces kluyveri (7 Dl and 13 Dl), it is possible to observe agglutination between opposite mating types of the same species:

    a. Culture the yeast strains separately on yeast extract-malt extract medium (3 gm. each of yeast extract and malt extract, 5 gm. of peptone, 10 gm. of glucose, and 20 gm. of agar per liter of distilled water -- the pH is not adjusted).

    b. After three or more days mix the opposite mating types on yeast-malt agar slants and observe the abundant formation of zygotes at the end of 2 hours.

    c. To demonstrate stronger agglutination in liquid culture inoculate the opposite mating types into separate flasks using the same medium without agar and with 3% glucose instead of 1%. Place on a shaker for 3 days, at 28° C.

    d. Pour the two flasks together into a graduate cylinder leaving considerable space above the liquid. Invert the cylinder about 20 times, and observe the occurrence of a strong agglutination reaction.

e. A similar agglutination may be demonstrated with opposite mating types of Hansenula wingei (5 and 21). Both S. kluyveri and H. wingei may show stronger agglutination after daily transfer for 3 or more days before this experiment is performed.

C. QUESTIONS:

1. Distinguish between the four families of the Endomycetales.
2. The Ascoideaceae are said to represent a link between the Ascomycetes and the Zygomycetes. Defend this viewpoint.
3. How does sexual reproduction take place in the yeasts? Asexual reproduction?
4. What is meant by ascosporogenous yeasts? Anascosporogenous yeasts?
5. Explain haplobiontic, diplobiontic, and haplo-diplobiontic life cycles in yeasts and name an organism which exhibits each of these types of life cycle.
6. Of what importance to man are the fungi included in the Endomycetales?
7. Explain the biochemical basis for mating in some yeasts (Brock, 1959).

D. TEXT REFERENCES:

Alexopoulos -- pp. 217-254.
Bessey -- pp. 334-353.

E. SELECTED REFERENCES:

1. BIGGS, ROSEMARY. 1937. Dipodascus uninucleatus. Mycologia, 29:34-44.
2. BROCK, T. D. 1959a. Biochemical basis of mating in yeast. Science, 129:960-961.
3. BROCK, T. D. 1959b. Mating reaction in Hansenula wingei. Relation of cell surface properties to agglutination. Jr. Bact., 78:59-68.
4. COOK, A. H. (Editor). 1958. The chemistry and biology of yeast. xii + 736 pp. Illustr. Academic Press, New York.
5. GAÜMANN, E. A. 1949. (English translation by F. L. Wynd.) The Fungi. Hafner Publishing Co., New York.
6. GUILLIERMOND, A. 1940. Sexuality, developmental cycles and phylogeny of the yeasts. Bot. Rev., 6:1-24.
7. HENRICI, A. T. 1941. The yeasts. Genetics, cytology, variation, classification and identification. Bact. Rev., 5:97-179.
8. LINDEGREN, C. C. 1949. The yeast cell, its genetics and cytology. viii + 28 chapters. Educational Publishers, Inc., St. Louis.
9. LINDEGREN, C. C. and G. LINDEGREN. 1943. Segregation, mutation and copulation in Saccharomyces cerevisiae. Ann. Mo. Bot. Gard., 30:453-464.
10. LINDEGREN, C. C. and G. LINDEGREN. 1943. Selecting, inbreeding, recombining and hybridizing commercial yeasts. Jr. Bact., 46:405-419.
11. LINDEGREN, C. C. and G. LINDEGREN. 1944. Sporulation in Saccharomyces cerevisiae. Bot. Gaz., 105:304-316.
12. LODDER, J., and N. J. W. KREGER-VAN RIJ. 1952. The yeasts. xi + 713 pp. 268 figs. North Holland Publishing Co., Amsterdam.
13. PRESCOTT, S. C. and C. G. DUNN. 1949. Industrial microbiology. McGraw-Hill Book Co., Inc. New York.
14. SKINNER, C. E. 1947. The yeast-like fungi: Candida and Brettanomyces. Bact. Reviews, 11:227-274.
15. SKINNER, C. E. 1950. Generic name for imperfect yeasts. Cryptococcus or Torulopsis? Am. Midl. Nat., 43:242-250.
16. SKINNER, C. E., and R. BOUTHILET. 1947. Melibiose broth for classifying yeasts. Jr. Bact., 53:37-43.
17. SKINNER, C. E., and C. W. EMMONS, and H. M. TSUCHIYA. 1947. Henrici's Molds, yeasts and actinomycetes. xii + 409 pp. John Wiley and Sons, Inc. New York.
18. SUBRAMANIAM, M. K. 1951. Tetraploidy in yeasts. La Cellule, 54:145-148.
19. WINGARD, S. A. 1925. Studies on the pathogenicity, morphology, and cytology of Nematospora phaseoli. Bull. Torrey Bot. Club, 52:249-290.
20. WICKERHAM, L. J. 1951. Taxonomy of yeasts. U. S. Dept. Agr. Tech. Bull. 1029. ii + 56 pp. 5 pls.
21. WICKERHAM, L. J. 1956. Influence of agglutination on zygospore formation in Hansenula wingei, a new species of yeast. Compt. rend. Lab. Carlsberg, Ser. Physiol., 26:423-443.
22. WICKERHAM, L. J. 1958. Sexual agglutination of heterothallic yeasts in diverse taxonomic areas. Science, 128:1504-1505.

F. GLOSSARY:

1. Ascocarp (Gr. askos = sac. + karpos = fruit): a fruiting body containing asci.
2. Ascogenous hypha (Gr. askos = sac + gennao = I give birth; hyphe = web): a specialized hypha which gives rise to asci.

Class Ascomycetes – Sub-Class Hemiascomycetidae

Order: Taphrinales

Family: Taphrinaceae

A. GENERAL REMARKS:

1. The Taphrinales are parasitic on plants. In culture they resemble the yeasts in that they develop yeast-like colonies composed of yeast-like cells which reproduce by budding.

2. In nature they grow intercellularly in the host forming binucleate mycelium. This breaks up into binucleate, chlamydospore-like cells from which the asci arise naked on the surface of the host, forming a hymenium.

B. PROCEDURE:

3. If fresh material is available section infected peach leaves and mount the sections in water. Study the asci of *Taphrina deformans*.

4. Study prepared, stained sections showing the hymenial layer with asci at different stages of development. Note the stalk cell, the asci and the ascospores.

5. If time permits study other species of *Taphrina*, and compare with *Taphrina deformans*.

C. QUESTIONS:

1. How does the binucleate condition arise in *Taphrina deformans*? In other species of *Taphrina*?
2. How does the fungus reproduce asexually?
3. How does this fungus overwinter?
4. Diagram the life cycle of *Taphrina deformans* based on the findings of Miss Martin (1940).
5. Name some other species of *Taphrina* which cause economically important plant diseases.

D. TEXT REFERENCES:

Alexopoulos -- pp. 255-258.
Bessey -- pp. 241-244.

E. SELECTED REFERENCES:

1. FITZPATRICK, R. E. 1934. The life history and parasitism of *Taphrina deformans*. Sci. Agr., 14:305-326.
2. KRAMER, C. L. 1960 (1961). Morphological development and nuclear behavior in the genus *Taphrina*. Mycologia, 52:295-320.
3. MARTIN, ELLA M. 1924. Studies on the morphology and cytology of *Taphrina coryli* Nashida. Trans. Wis. Acad. Sci., 21:345-356.
4. MARTIN, ELLA M. 1925. Cultural and morphological studies of some species of *Taphrina*. Phytopath., 15:67-76.
5. MARTIN, ELLA M. 1940. The morphology and cytology of *Taphrina deformans*. Am. Jr. Bot., 27:743-751.
6. MIX, A. J. 1949. A monograph of the genus *Taphrina*. University of Kansas Sci. Bull. Vol. 33, pt. 1, No. 1, 167 pp.
7. ROBERTS, CATHERINE. 1946. A comparative study of *Torulopsis pulcherina* and *Taphrina deformans* in culture. Farlowia, 2:345-383.

F. GLOSSARY:

1. Hymenium (Gr. hymen = membrane): a layer of asci or basidia.

Class Ascomycetes – Sub-Class Euascomycetidae – Series Plectomycetes

A. GENERAL REMARKS:

1. The sub-class Euascomycetidae embraces all the Ascomycetes which produce unitunicate asci from ascogenous hyphae in a fruiting body called the ascocarp. In some lichen fungi the asci are bitunicate.

2. The Euascomycetidae are conveniently classified into four series: the Plectomycetes, the Pyrenomycetes, the Discomycetes, and the Laboulbeniomycetes.

3. Fungi in the series Plectomycetes characteristically bear their typically globose or broadly ovate asci at various levels in a completely closed ascocarp (cleistothecium).

Order: Eurotiales

A. GENERAL REMARKS:

4. The Eurotiales have freely branched mycelium, and are found frequently on decaying fruits, meats, organic matter in the soil, and a wide variety of other organic substrata.

5. Conidia are produced in great numbers on conidiophores, in asexual reproduction. Asci are formed sexually from ascogenous hyphae which originate on the ascogonium. The completely closed ascocarp is composed of interwoven hyphae, and the globose asci are irregularly distributed within the ascocarp. The asci are evanescent, dissolving away and shedding the ascospores in the cleistothecial cavity.

6. The Eurotiales are subdivided into three families; these are: the Ascosphaeriaceae, the Gymnoascaceae, and the Eurotiaceae.

B. PROCEDURE:

Family: Gymnoascaceae

Genera: Arachniotus, Arthroderma, Ctenomyces, Gymnoascus, Myxotrichum, Nannizzia

1. The Gymnoascaceae include fungi which often grow on animal substrata such as feathers, nails, hair, etc. Many are also developed on animal dung. The genera Arthroderma and Nannizzia include the ascus stages of the dermatophytes.

2. Obtain rabbit dung or other animal dung and place in a moist chamber for several days. Observe periodically for the appearance of loose, cottony ascocarps. In many genera these bear characteristic appendages. Mount such an ascocarp in lactophenol, heat the slide gently and examine under the microscope. With the help of a good key (Benjamin, 1956) identify the genus in each case. Compare with known cultures.

3. Place some soil of high organic content in a Petri dish and moisten with sterile, distilled water. Scatter short, sterilized clippings of human or animal hair on the soil. Incubate for four to six weeks at room temperature and examine for cleistothecia of Nannizzia and Arthroderma. The former includes the perfect stages of some species of Microsporum and the latter of some species of Trichophyton and Keratinomyces. All these are dermatophytes.

Family: Eurotiaceae

Genera: Emericella, Eurotium, Sartorya (all linked with the form-genus Aspergillus)

4. There are a number of species of Aspergillus important economically in the production of organic acids. A few are pathogenic to man and animals.

5. Examine colonies of various species of the above genera and make a thorough study of the somatic, asexual, and sexual phases, as follows:

   a. Examine the well-developed, septate hyphae. The manner of hyphal growth on the surface of the substratum and the pigmentation are characteristics used as aids in identification of species.

   b. Make a water mount of a small thin strip of the colony. A razor blade or sharp scalpel may be used to cut such a strip. Mount the strip on its side and examine the structure of the hyphae and the aerial conidiophores. Note the following structures:

      (1) Stalk cell (also called foot cell)
      (2) Vesicle
      (3) Sterigmata, one or two rows
      (4) Chains of conidia

   c. Examine other species of Aspergillus. Compare the shape of the conidial heads; note differences in color, colony characteristics, shape and surface markings of the conidiophores and conidia, and number of rows of sterigmata.

   d. Study cultures on Czapek's agar which bear cleistothecia characteristic of each of the three genera (Eurotium, Sartorya, and Emericella) which produce Aspergillus imperfect stages.

   e. Place a cover-glass over the younger parts of a six-day old colony and press gently until it is in intimate contact with the agar. A small drop of water placed on the colony before the cover-glass is dropped may help avoid air bubbles. Search the covered spot under the high power objective and find the ascogonial coils at different stages of development. Find young cleistothecia which are developing.

   f. Examine a colony 9-10 days old. Mount a large cleistothecium in water under a cover-glass, break it by applying pressure, and find the asci with the ascospores.

   g. If mature cleistothecia are present, examine the mature ascospores under the oil immersion objective and note their shape. Note that the asci have evanesced by this time.

   h. Examine prepared slides showing cross sections of cleistothecia with the irregularly arranged asci. Note the shape of the ascus and the ascospores. The wall of the cleistothecium consists of more or less loosely interwoven hyphae.

Genera: Carpenteles, Tallaromyces (linked with the form-genus Penicillium)

6. Many species of Penicillium are economically important. Some cause fruit rots and some are used industrially in the production of antibiotics and of certain cheeses.

7. Repeat the study outlined under paragraphs 5 a - h for the above two genera. Note that the tip of the conidiophore resembles a broom rather than a bulbous head. This is the penicillus, commonly called a "brush". Examine the manner of hyphal growth and the pigmentation in the cultures. Note that the conidiophores are erect in common with those of Aspergillus.

8. Mount some fruit tissue from the edge of an infected portion of an orange or other fruit with soft rot caused by Penicillium. Study the relationship between the host cells and the fungous hyphae. Penicillium digitatum develops a soft rot on oranges, and produces olive-green conidia over the surface of the fruit. P. italicum also attacks citrus fruits forming blue-green conidial masses. P. expansum produces a similar rot on apples and other fruits.

C. QUESTIONS:

1. Characterize the Plectomycetes.
2. Characterize the Eurotiales.
3. In what connection do we use the names Aspergillus and Penicillium?
4. Name at least six ways in which Aspergillus and Penicillium are of importance to man.
5. How does Penicillium resemble Aspergillus? How does it differ?
6. How would you proceed to isolate Aspergillus and Penicillium from natural substrata?
7. Describe the formation of conidia in Aspergillus and in Penicillium.
8. What are "hülle" cells?
9. Discuss the evolution of sexual reproduction in the Eurotiaceae.

D. TEXT REFERENCES:

Alexopoulos -- pp. 262-268, 269-283.
Bessey -- pp. 322-331.

E. SELECTED REFERENCES:

1. CRICKSHANK, J. H., H. RAISTRICK, and R. ROBINSON. 1938. The chemistry of Aspergillus colouring matters. Part II. (London) Chem. Soc. Jour., 1938:2056-2064.
2. DALE, E. 1909. On the morphology and cytology of Aspergillus repens de Bary. Ann. Mycol., 7:215-225.
3. DAWSON, CHRISTINE O., and J. C. GENTLES. 1961. The perfect state of Keratinomyces ajelloi van Breuseghem, Trichophyton terrestre Durie ana Frey, and Microsporum nanum Fuentes. Sabouraudia, 1:49-57.
4. EMMONS, C. W. 1935. The ascocarps of species of Penicillium. Mycologia, 27:122-150.
5. EMMONS, C. W. 1954. Isolation of Myxotrichum and Gymnoascus from the lungs of animals. Mycologia, 46:334-338.
6. FENNELL, DOROTHY I., and J. H. WARCUP. 1959. The ascocarps of Aspergillus alliaceus. Mycologia, 51:409-415.
7. FRAZER, HELEN C. I. and H. S. CHAMBERS. 1907. The morphology of Aspergillus herbariorum. Ann. Mycol., 5:419-431.
8. GÄUMANN, E. A. 1949. (English transl. by F. L. Wynd, in press.) The Fungi. Hafner Publishing Co. New York.
9. KUEHN, H. H. 1960. Observations on Gymnoascaceae. VIII. A new species of Arthroderma. Mycopath. et Mycol. Appl., 13:189-197.
10. OLIVE, L. S. 1944. Development of the perithecium of Aspergillus Fischeri Wehmer, with a description of crozier formation. Mycologia, 36:266-275.
11. RAPER, K. B. 1957. Nomenclature in Aspergillus and Penicillium. Mycologia, 49:644-662.
12. RAPER, K. B. and DOROTHY I. FENNELL. 1952. Homothallism vs. heterothallism in the Penicillium luteum series. Mycologia, 44:101-111.
13. RAPER, K. B., D. I. FENNELL, and H. D. TRESNER. 1953. The ascosporic stage of Aspergillus citrisporus and related forms. Mycologia, 45:671-692.

14. RAPER, K. B. and C. THOM. 1949. A manual of the penicillia. iv + 875 pp. The Williams and Wilkins Co. Baltimore.
15. SKINNER, C. E., C. W. EMMONS, and H. M. TSUCHIYA. 1947. Molds, yeasts and actinomycetes. xiv + 409 pp. John Wiley and Sons, Inc. New York.
16. STOCKDALE, PHYLLIS M. 1961. Nannizzia incurvata gen. nov., sp. nov., a perfect stage of Microsporum gypseum (Bodin, Guiart, et Grigorakis). Sabouraudia, 1:41-48.
17. THOM, C. 1954. The evolution of species concepts in Aspergillus and Penicillium. Ann. N. Y. Acad. Sci., 60:24-34.
18. THOM, C. and K. B. RAPER. 1945. A manual of the aspergilli. ix + 373 pp. The Williams and Wilkins Co. Baltimore.
19. YUILL, E. 1950. The numbers of nuclei in conidia of aspergilli. Trans. Brit. Mycol. Soc., 33:324-331.

F. GLOSSARY:

1. Cleistothecium (pl. cleistothecia; Gr. kleistos = closed + theke = box): a completely closed ascocarp.
2. Dermatophyte (Gr. derma = skin + phyton = plant): anyone of several fungi which cause skin diseases.
3. Penicillus (pl. penicilli; L. penicillum = small brush): the conidiophore of the genus Penicillium.

Class Ascomycetes – Sub-Class Euascomycetidae – Series Pyrenomycetes

A. GENERAL REMARKS:

1. The Pyrenomycetes bear their unitunicate asci in a basal layer (hymenium) or in one or more basal tufts, inside a more or less closed ascocarp.

2. The typical ascocarp of the Pyrenomycetes is the <u>perithecium</u>. A perithecium is a more or less globose or flask-shaped, hollow structure whose wall originates below the ascogonium and grows around the developing asci. The upper portion of the perithecial wall forms a papilla or a neck, which is lined inside by a fringe of hairs, the <u>periphyses</u>, and which is provided with a pore through which the ascospores are expelled forcibly. This neck-like structure is called the <u>ostiole</u>. The perithecium may or may not be surrounded by stromatic tissue.

3. The ascocarp of a few Pyrenomycetes is entirely stromatic, lacking a true perithecial wall, and bears its unitunicate asci in one or more cavities (locules) within the stroma in the manner of the Loculoascomycetidae (page 146). Such an ascocarp develops a pore at maturity over each cavity, through which the ascospores are ejected.

4. As we shall see presently, in some Pyrenomycetes the ascocarp is a cleistothecium, being completely closed.

Order: Erysiphales

5. The ascocarp of the Erysiphales is completely closed (cleistothecium). Some mycologists, consequently, prefer to classify such forms with the Plectomycetes in spite of the fact that the asci are not scattered within the cleistothecium as they are in the typical Plectomycetes.

6. The cleistothecia of the Erysiphales have a <u>pseudoparenchymatous</u> wall which generally bears long appendages or bristles of various types. The asci are globose or broadly oval and arise from the base of the ascocarp. The mycelium is superficial in all but a few species, and obtains nourishment by means of haustoria. The Erysiphales are specialized parasites of plants.

7. The order Erysiphales is subdivided into a number of families of which the Erysiphaceae are the most widely distributed and the best known.

B. PROCEDURE:

Family: Erysiphaceae

Genera: Erysiphe, Sphaerotheca, Microsphaera, Uncinula, Phyllactinia, Podosphaera

8. <u>Somatic hyphae</u>. The mycelium of the Erysiphaceae is colorless. The hyphae obtain nourishment by means of haustoria which invade the epidermal cells of the host. Using a dissecting microscope, examine living leaves or stems of a herbaceous plant such as bean or strawberry, infected with a powdery mildew. Note

the hyphae which form a white mat over the infected portions. Strip a portion of the epidermis and mount in water. Study the hyphae of the fungus.

9. Conidial stage. Observe the erect conidiophores and the conidial chains under the high power of the dissecting microscope. Study these structures under the compound microscope in a water mount. If you cannot find conidia still attached to the conidiophores cut a very thin section of the infected leaf, place it on a clean slide and examine it under the microscope as a dry mount without a cover-slip. Study the conidial stages of two or more genera and compare.

10. Ascigerous stage: Mount some cleistothecia in water or lacto-phenol under a cover-glass. Note the appendages characteristic of the genus, paying particular attention to the tips and bases of the appendages, and to the manner in which the tips may be branched. While looking through the tube of the microscope press on the cover-glass with a mounted needle and watch the asci with ascospores break out of the cleistothecia.

11. Study the ascigerous stages of as many genera as are made available to you and note the structures which will enable you to distinguish the genera from each other.

C. QUESTIONS:

1. Describe the relations of the somatic hyphae of the Erysiphaceae to their hosts.
2. What correlation exists between the Erysiphaceae and their conidial forms which is not very common in other ascomycetous families?
3. Construct a key to the North American genera of the Erysiphaceae.
4. Name at least one plant disease caused by a member of each of these genera and give the name of the causal organism in each case.
5. Draw a life cycle diagram typifying the Erysiphaceae.
6. Discuss ascocarp formation in the Erysiphaceae.
7. What do we know about homothallism and heterothallism in the Erysiphaceae?
8. Is it possible to culture the Erysiphaceae in the laboratory? Explain.
9. Discuss physiological specialization in the Erysiphaceae.

D. TEXT REFERENCES:

Alexopoulos -- pp. 292-300.
Bessey -- pp. 307-317.

E. SELECTED REFERENCES:

1. CHEREWICK, W. J. 1944. Studies on the biology of Erysiphe graminis DC. Can. Jr. Res. C., 22:62-86.
2. CHIDDARWAR, P. P. 1959. Salmonomyces -- a new member of the Erysiphaceae. Sydowia, Ann. Mycol., 13:55-56.
3. COLSON, BARBARA. 1938. The cytology and development of Phyllactinia corylea. Lev. Ann. Bot., n. s. 2:381-402.
4. HEIM, JEAN M. and G. H. GREIS. 1953. The culture of Erysiphe cichoracearum on sunflower tumor tissue. Phytopath,, 43:343-344.
5. HEIN, I. 1927. Studies on morphogenesis and development of the ascocarp of Sphaerotheca castagnei. Bull. Torrey Bot. Club, 54:383-417.
6. MAINS, E. B. and S. M. DIETZ. 1930. Physiologic forms of barley mildew Erysiphe graminis hordei Marchal. Phytopath., 24:229-239.
7. MILLER, J. H. 1949. A revision of the classification of the Ascomycetes with special emphasis on the Pyrenomycetes. Mycologia, 41:99-127.
8. MORRISON, R. M. 1960 (1961). Studies of clonal isolates of Erysiphe cichoracearum on leaf disk culture. Mycologia, 52:388-293.
9. REED, G. M. 1913. The powdery mildews - Erysiphaceae. Trans. Am. Micr. Soc., 32:219-258.
10. SALMON, E. S. 1900. A monograph of the Erysiphaceae. Mem. Torrey Bot. Club, 9:1-292.
11. SALMON, E. S. 1906. On Oidiopsis taurica Lev., an endophytic member of Erysiphaceae. Ann. Bot., 20:187-200.
12. SCHMITT, J. A., JR. 1955. The host specialization of Erysiphe cichoracearum from Zinnia, Phlox, and cucurbits. Mycologia, 47:688-701.

13. SCHMITT, J. A. 1957. Comparative morphology of the Zinnia, Phlox, and cucurbit powdery mildews. Am. Jr. Bot., 44:120-125.
14. SCHNATHORST, W. C. 1959a. Heterothallism in the lettuce strain of Erysiphe cichoracearum. Mycologia, 51:708-711.
15. SCHNATHORST, W. C. 1959b. Growth of Erysiphe cichoracearum on isolated lower epidermis and spongy mesophyll of lettuce. Phytopath., 49:115-116.
16. SCHNATHORST, W. C. 1959c. Spread and life cycle of the lettuce powdery mildew fungus. Phytopath., 49:464-468.
17. SMITH, G. 1900. The haustoria of the Erysiphaceae. Bot. Gaz., 29:153-184.
18. YARWOOD, C. E. 1936. The diurnal cycle of the powdery mildew Erysiphe polygoni. Jour. Agr. Res., 52:645-657.
19. YARWOOD, C. E. 1957. Powdery mildews. Bot. Rev., 23:235-300.

F. GLOSSARY:

1. Ostiole (L. ostiolum = little door): a neck-like structure in an ascocarp lined with periphyses and terminating in a pore. Also the opening of a pycnidium or a spermogonium.
2. Periphyses (sing. periphysis; Gr. peri = around + physis = a growth): short, hair-like growths in the form of a fringe lining the inside of an ostiole or of a pore in a stroma.
3. Perithecium (pl. perithecia; Gr. peri = around + theke = case): a globose, hollow ascocarp whose wall originates below the ascogonium, grows around the developing asci, and terminates at the top into a neck-like structure provided with a round pore.
4. Pseudoparenchyma (Gr. pseudo = false + parenchyma = a type of plant tissue): a type of plectenchyma (fungal tissue) consisting of oval or isodiametric cells, the component hyphae having lost their individuality.
5. Unitunicate (L. unus = one + tunica = coat, mantle): an ascus in which both the inner and outer wall are more or less rigid and do not separate during spore ejection.

Class Ascomycetes – Sub-Class Euascomycetidae – Series Pyrenomycetes

Order: Chaetomiales

A. GENERAL REMARKS:

1. The Chaetomiales, a relatively small order of Pyrenomycetes, have only recently been segregated from the Sphaeriales.

2. These cellulolytic fungi are easily recognized by the numerous long hairs on the superficial, non-stromatic perithecia. In many species the hairs on the upper part of the perithecium are conspicuously curly giving the ascocarp a characteristic appearance.

3. The asci, borne in basal tufts, are evanescent at an early stage. Because of this character, some mycologists consider the Chaetomiales to be closely related to the Plectomycetes.

4. The single family Chaetomiaceae includes 3 genera: *Chaetomium*, *Ascotricha*, and *Lophotrichus*, in accordance with the excellent monograph by Lawrence Ames. The first of these genera is the largest by far and the most commonly encountered.

B. PROCEDURE:

Family: Chaetomiaceae

Genus: Chaetomium

5. Using a good stereomicroscope, examine perithecia of *Chaetomium* on paper, cloth, or other substratum on which they have been formed. Note their characteristic appearance and learn to recognize them at sight.

6. Study the hyphae of any species of *Chaetomium* growing in agar culture.

7. Mount perithecia of several species of *Chaetomium* and compare the structure of the hairs under the compound microscope.

8. Crush a mature perithecium and find the numerous dark ascospores. In many species these are characteristically lemon-shaped.

9. Crush a young perithecium from agar culture and find the asci with developing ascospores. Study the origin of the asci. Note their shape.

10. If *Ascotricha* and *Lophotrichus* cultures are available, compare these two genera with *Chaetomium*.

C. QUESTIONS:

1. Discuss the economic importance of the Chaetomiales.
2. What is the characteristic shape of the asci in *Chaetomium*? Is this true of all species in the genus?

3. How are the asci arranged?
4. Why do you not find asci in mature perithecia? How do you know such structures are perithecia?
5. How are the ascospores released from the ascocarps?
6. Does Chaetomium have a conidial stage? Ascotricha? Lophotrichus?
7. Where does Bessey (1950) classify the Chaetomiaceae? Lutrell (1951)? Gäumann (1952)? Martin (1961)? Discuss the various viewpoints.

D. TEXT REFERENCES:

Alexopoulos -- pp. 301-302.
Bessey -- pp. 275.

E. SELECTED REFERENCES:

1. AMES, L. M. (In Press). A monograph of the Chaetomiaceae.
2. BASU, S. N. and R. G. BOSE. 1950. Factors affecting the fruiting of Chaetomium species. Jr. Gen. Microbiol., 4:132-140.
3. BENJAMIN, R. K. 1949. Two species representing a new genus of the Chaetomiaceae. Mycologia, 41:346-354.
4. BUSTON, H. W. and B. RICKARD. 1956. The effect of a physical barrier on sporulation of Chaetomium globosum. Jr. Gen. Microbiol., 15:194-197.
5. CHIVERS, A. H. 1915. A monograph of the genera Chaetomium and Ascotricha. Mem. Torrey Bot. Club, 14:155-240.
6. GÄUMANN, E. A. 1952. The fungi. (Transl. by F. L Wynd.) 420 pp., 440 figs. Hafner Publishing Co., New York.
7. LILLY, V. G. and H. L. BARNETT. 1949. The influence of nutrients, thiamin, and biotin upon the growth and formation of perithecia and ascospores by Chaetomium convolutum. Mycologia, 41:186-196.
8. LUTTRELL, E. S. 1951. Taxonomy of the pyrenomycetes. Univ. of Missouri Studies 24, No. 3, 120 pp. Curators Univ. of Missouri, Columbia.
9. MARTIN, G. W. 1961. Key to the families of fungi. In Ainsworth's Dictionary of the fungi. Pp. 497-517. Commonwealth Mycol. Inst., Kew.
10. SKOLKO, A. J. and J. W. GROVES. 1948. Notes on seed-borne fungi. V. Chaetomium species with dichotomously branched hairs. Can. Jr. Res. C, 26:269-280.
11. SKOLKO, A. J. and J. W. GROVES. 1953. Notes on seed-borne fungi. VII. Chaetomium. Can. Jr. Bot., 31:779-809.
12. VAN DER WEYEN, A. 1954. L'évolution nucléaire et les hyphes ascogénes chez Chaetomium globosum Kunze. La Cellule, 56:213-225.

Class Ascomycetes – Sub-Class Euascomycetidae – Series Pyrenomycetes

Order: Sphaeriales (Xylariales)

A. GENERAL REMARKS:

1. The Sphaeriales, also called Xylariales, constitute one of the larger orders of Pyrenomycetes. Their perithecia are usually dark brown or black, leathery or carbonous and may or may not be associated with a stroma. A stroma (pl. stromata) is a cushion-like structure on or in which reproductive bodies are produced.

2. Paraphyses are interspersed with the asci in the early stages of perithecial development, but may be completely absent from the mature ascocarps. The asci are cylindrical or clavate and are arranged in a hymenial layer. They are provided with a pore through which the ascospores are forcibly expelled or ooze out.

3. The number of families into which the Sphaeriales are divided varies with the author. Miller (1949) recognizes 5 families; Dennis (1960), 14; Martin (1961), 4.

B. PROCEDURE:

Family: Sordariaceae

4. The Sordariaceae are either dung-inhabiting or occur on decaying plant parts. The perithecia are typically beaked and are covered, at least partially, with soft hairs.

5. The spores are dark brown to black and are variously ornamented. Some bear gelatinous appendages. The character of the spores is a major criterion for identification.

Genera: Sordaria, Podospora (Pleurage), Neurospora, Gelasinospora

6. Study the perithecia, asci, and ascospores of as many genera in this family as are made available to you and familiarize yourself with the characteristics of each.

7. Mount some asci in iodine (Lugol's solution) and study the structure of the ascal apex.

8. Examine self-fertile (homothallic) and self-sterile (heterothallic) monosporous cultures of the same genus as well as paired self-sterile inter-fertile strains and note the pattern of perithecial production.

9. Note incidence of ascospore abortion, giant spores, dwarf spores, and other characteristic abnormalities which may occur in your cultures.

10. Using the procedure recommended by Tylutki (1958) isolate single ascospores of Gelasinospora calospora var. autosteira and grow them separately in test tubes on corn meal agar. When the cultures have become established, mate them in all possible combinations in Petri dishes and observe perithecial production.

11. Observe the microconidiophores and the microconidia of this organism under the oil immersion objective. (See Goos, 1959.)

Family: Phyllachoraceae

Genus: Phyllachora

12. Collect grass leaves infected with Phyllachora graminis, a common parasite of many grasses. Note the characteristic black streaks on the infected leaves. You are most likely to find mature asci and ascospores of the fungus in early spring on overwintered dead grass leaves.

13. Cut cross sections of the leaf through an infected region and find the perithecia. Observe the asci and the ascospores. Note the large number of paraphyses present. These absorb water, expand, and in so doing help distribute the asci and ascospores. Note the clypeus above the perithecia. No conidial stage has been found for this fungus.

Family: Xylariaceae

Genera: Daldinia, Hypoxylon, Nummularia, Rosellinia, Xylaria

14. The shape of the stroma is one of the chief characters on which the various genera of the Xylariaceae are separated. Examine several types of stromata and become familiar with the various types.

15. Cut as thin a cross-section of a stroma of Hypoxylon coccineum as you can, using a sharp razor blade, and study the perithecia which are embedded therein. Note the asci and the ascospores.

16. Young stromata of Hypoxylon bear conidia on their surface. Study the conidiophores and the conidia.

17. Repeat your studies with Xylaria, Daldinia, Nummularia and Rosellinia.

C. QUESTIONS:

1. Construct a dichotomous key to the families of the Sphaeriales.
2. Discuss Miller's classification of the Sphaeriales and his interpretation of the ascocarp structure.
3. What are paraphyses and what is their function? How do they differ from periphyses?
4. Discuss "heterothallism" in the Ascomycetes as exemplified by Neurospora sitophila. How does it differ, if at all, from that in Rhizopus stolonifer?
5. Discuss sexual reproduction in the genus Gelasinospora.
6. What is a stroma? Of what elements may stromata be composed?
7. Discuss the taxonomy of the Phyllachoraceae (Consult Wolf and Wolf, Miller, Orton, and Bessey).
8. Discuss the importance of the Sphaeriales using specific examples to illustrate your points.

D. TEXT REFERENCES:

1. Alexopoulos -- pp. 302-313.
2. Bessey -- pp. 262-281.

E. SELECTED REFERENCES:

1. ALEXOPOULOS, CONST. J. and SUNG HUANG SUN. 1950. A new species of Gelasinospora. Mycologia, 42:723-734.
2. AMES, L. M. 1934. Hermaphroditism involving self-sterility and cross-fertility in the ascomycete Pleurage anserina. Mycologia, 26:392-414.
3. AMES, L. M. 1942. An hermaphroditic self-sterile but cross-fertilized condition in Pleurage anserina. Bull. Torrey Bot. Club, 59:341-345.
4. ARNOLD, C. A. 1928. The development of the perithecium and spermogonium of Sporormia leporina Niesst. Am. Jr. Bot., 15:241-245.
5. BACKUS, M. P. 1939. The mechanics of conidial fertilization in Neurospora sitophila. Bull. Torrey Bot. Club, 66:63-76.
6. BAKERSPIGEL, A. 1959a. The structure and manner of division of the nuclei in the vegetative mycelium of Neurospora crassa. Am. Jr. Bot., 46:180-190.
7. BAKERSPIGEL, A. 1959b. The structure and manner of division of the nuclei in the vegetative mycelium of Gelasinospora tetrasperma Dowd. Can. Jr. Microbiol., 5:125-130.
8. BARNETT, H. L. and V. G. LILLY. 1947. The effects of biotin upon the formation and development of perithecia, asci and ascospores by Sordaria fimicola Ces. and de Not. Am. Jr. Bot., 34:196-204.
9. BEADLE, G. W. 1945. Biochemical genetics. Chem. Rev., 37:15-96.
10. BEADLE, G. W. and V. L. COONRADT. 1944. Heterokaryosis in Neurospora crassa. Genetics, 29:291-308.
11. BERGMAN, B. 1941. Zytologische Studien über die Befruchtung und Askokarpbildung bei Sphaerotheca castagnei Lev. Svensk Botan. Tid., 35:194-210.
12. BRETZLOFF, C. W., JR. 1954. The growth and fruiting of Sordaria fimicola. Am. Jr. Bot., 41:58-67.
13. BUTLER, E. F. 1956. Spore germination in Sordaria fimicola. Mycologia, 48:345-348.
14. CAIN, R. F. 1934. Studies of coprophilous Sphaeriales in Ontario. Univ. of Toronto Studies, Biological Series No. 38, 126 pp.
15. CAIN, R. F. 1950. Studies of coprophilous Ascomycetes. I. Gelasinospora. Can. Jr. Res., C. 28:566-576.
16. CAIN, R. F. 1956. Studies of coprophilous Ascomycetes. IV. Tripterospora, a new cleistocarpous genus in a new family. Can. Jr. Bot., 34:699-710.
17. CAIN, R. F. 1957. Studies of coprophilous Ascomycetes. VI. Species from the Hudson Bay area. Can. Jr. Bot., 35:255-268.
18. CAIN, R. F. 1962. Studies of coprophilous Ascomycetes. VIII. New species of Podospora. Can. Jr. Bot., 40:447-490.
19. CAIN, R. F. and J. W. GROVES. 1948. Notes on seed borne fungi. VI. Sordaria. Can. Jr. Res., C26:486-495.
20. CARR, A. J. H. and L. S. Olive. 1958. Genetics of Sordaria fimicola. II. Cytology. Am. Jr. Bot., 45:142-150.
21. CARR, A. J. H. and L. S. OLIVE. 1959. Genetics of Sordaria fimicola. III. Cross-compatibility among self-sterile and sefl-fertile cultures. Am. Jr. Bot., 46:81-91.
22. DODGE, B. O. 1932. The non-sexual and the sexual functions of macroconidia of Neurospora. Bull. Torrey Bot. Club, 59:347-360.
23. DODGE, B. O. 1935. The mechanics of sexual reproduction in Neurospora. Mycologia, 27:418-438.
24. DOGUET, G. 1960. Contribution a l'étude de noyau du Sordaria fimicola. Rev. Cytol. Biol. Veg., 22:109-130.
25. DOWDING, E. S. 1933. Gelasinospora, a new genus of Pyrenomycetes with pitted spores. Can. Jr. Res., 9:294-305.
26. DOWDING, ELEANOR S. and A. H. R. BULLER. 1940. Nuclear migration in Gelasinospora. Mycologia, 32:471-488.
27. DOWDING, ELEANOR S. and A. BAKERSPIGEL. 1954. The migrating nucleus. Can. Jr. Microbiol, 1:68-78.
28. ELLIS, J. J. 1960 (1961). Plasmogamy and ascocarp development in Gelasinospora calospora. Mycologia, 52:557-573.
29. EMERSON, M. R. 1948. Chemical activation of ascospore germination in Neurospora crassa. Jr. Bact., 55:327-330.
30. ESSER, K. 1954. Sur le determinisme génétique d'un nouveau type d'incompatibilité chez Podospora. Comptes. Rend., 237:666-668.
31. ESSER, K. 1958. The significance of semi-incompatibility in the evolution of geographical races in Posospora anserina. Prox. C Int. Cong. Gen., 2:76-77.
32. GILLES, A. 1950. Évolution nucléaire chez Neurospora tetrasperma. La Cellule, 53:395-415.
33. GOOS, ROGER D. 1959. Spermatium-trichogyne relationship in Gelasinospora calospora var. autosteira. Mycologia, 51:416-428.
34. GRIFFITHS, D. 1901. The North American Sordariaceae. Torrey Bot. Club Mem. No. 11, 134 pp. 6 figs. 19 pls.
35. GRIFFITHS, D. and F. J. SEAVER. 1910. Fimetariaceae. North American Flora. Vol. 3, pt. 1, pp. 65-88.
36. HACKBARTH, R. D. and R. P. COLLINS. 1961. A study of the vitamin requirements of three species of the genus Gelasinospora. Am. Jr. Bot., 48:603-606.
37. HAWKER, L. E. 1951. Morphological and physiological studies on Sordaria destruens (Shear) comb. nov. (Syn. Melanospora destruens), Sordaria fimicola and Melanospora zamiae. Trans. Br. Mycol. Soc., 34:174-186.
38. INGOLD, C. T. 1960. Spore discharge in Pyrenomycetes. Friesia, 6:148-163.
39. KHAN, A. H. 1959. Biology and pathogenicity of Rosellinia necatrix (Hart.) Berl. Biologia (Lahore), 5:199-245.
40. LILLY, V. G. and H. L. BARNETT. 1947. The influence of pH and certain growth factors on mycelial growth and perithecial formation by Sordaria fimicola. Am. Jr. Bot., 34:131-138.
41. LUTTRELL, E. S. 1951. Taxonomy of the Pyrenomycetes. Univ. Missouri Studies 24, No. 3, 120 pp. Univ. of Missouri, Columbia.
42. MAINS, E. B. and S. M. DIETZ. 1930. Physiologic forms of barley mildew Erysiphe graminis hordei Marchal. Phytopath., 24:229-239.
43. MC CLINTOCK, BARBARA. 1945. Neurospora I. Preliminary observations of the chromosomes of Neurospora crassa. Am. Jr. Bot., 32:671-678.
44. MEYER, J. 1957. Évolution nucléaire et organogénese chez Gelasinospora calospora (Mont.) Moreau. La Cellule, 58:345-362.
45. MILLER, J. H. 1949. A revision of the classification of the Ascomycetes with special emphasis on the Pyrenomycetes. Mycologia, 41:99-127.

46. MILLER, J. H. 1951. Studies in the Phyllachoraceae. I. Phyllachora ambrosiae (Berk. and Curt.) Sacc. Am. Jr. Bot., 38:830-834.
47. MILLER, J. H. 1954. Studies in the Phyllachoraceae. II. Phyllachora lespedezae. Am. Jr. Bot., 41:825-828.
48. MOREAU, C. and MOREAU, M. 1951. Le genre Gelasinospora Dowding. La Mycothèque. Prem. Suppl. Micromycètes, 39-41.
49. MOREAU, C. 1953. Les genres Sordaria et Pleurage. Encyclop. Mycol., 25:1-330.
50. MOREAU-FROMENT, MIREILLE. 1956. Les Neurospora. Bull. de la Soc. Bot. Fr., 103:678-738.
51. MORRISON, R. M. 1960 (1961). Studies of clonal isolates of Erysiphe cichoracearum on leaf disk culture. Mycologia, 52:388-393.
52. MUNK, A. 1953. The system of pyrenomycetes. Dansk Bot. Arkiv., 15:1-163.
53. MUNK, A. 1957. Danish pyrenomycetes. Dansk. Bot. Arkiv., 17:1-491.
54. NAKAMURA, K. and T. EGASHIRA. 1961. Genetically mixed perithecia in Neurospora. Nature (London), 190:1129-1130.
55. OLIVE, L. S. 1956. Genetics of Sordaria fimicola. I. Ascospore color mutants. Am. Jr. Bot., 43:97-106.
56. OLIVE, L. S. and A. A. FANTINI. 1961. A new, heterothallic species of Sordaria. Am. Jr. Bot., 48:124-128.
57. ORTON, C. R. 1944. Graminicolons species of Phyllachora in North America. Mycologia, 36:18-53.
58. RITCHIE, D. 1937. The morphology of the perithecium of Sordaria fimicola (Rob.) Ces. and de Not. Jr. El. Mitchell Sci. Soc., 53:334-342.
59. RIZET, G. and C. ENGELMANN. 1949. Contribution a l'étude génétique d'un ascomycete tetrasporé: Podospora anserina (Ces.) Rehm. Rev. Cytol. et Biol. Veget., 11:201-304. English summary.
60. SHEAR, C. L. and B. O. DODGE. 1927. Life histories and heterothallism of the red bread-mold fungi of the Monilia sitophila group. Jr. Agr. Res., 34:1019-1042.
61. SINGLETON, J. R. 1953. Chromosome morphology and the chromosome cycle in the ascus of Neurospora crassa. Am. Jr. Bot., 40:124-144.
62. SLOAN, B. J. and G. B. WILSON. 1958. The functions of the microspores of Gelasinospora calospora var. autosteira. Mycologia, 50:111-116.
63. TYLUTKI, E. E. 1958. Some aspects of morphology, genetics, and cultural behavior of Gelasinospora calospora var. autosteira. Mycologia, 50:333-356.
64. WESTERGAARD, M. and H. K. MITCHELL. 1947. Neurospora V. A synthetic medium favoring sexual reproduction. Am. Jr. Bot., 34:573-577.
65. ZICKLER, H. 1953. Zur Entwicklusgeschichte des Askomyzeten Bombardia lunata Zckl. Arch. Protistenk, 98:1-70.

F. GLOSSARY:

1. Clypeus (pl. clypei; L. clypeus = shield): a disc- or shield-like structure surrounding the mouth of the ascocarp in certain Ascomycetes.
2. Paraphyses (sing. paraphysis; Gr. para = beside + physis = growth): sterile, basally attached and apically free structures in a hymenium.
3. Stroma (pl. stromata; Gr. stroma = mattress): a compact, mattress-like somatic structure, on or in which fructifications are usually formed.

Class Ascomycetes – Sub-Class Euascomycetidae – Series Pyrenomycetes

Order: Diaporthales

A. GENERAL REMARKS:

1. The fungi included here are placed in the Sphaeriales by some authors (Bessey, 1950; Dennis, 1960). As delimited here, the Diaporthales are Sphaeriales-like fungi in which the stalks of the asci gelatinize, freeing the asci from the hymenial layer. The free asci thus fill the perithecial cavity and eventually issue from the perithecial ostiole.

2. The perithecial centrum is at first pseudoparenchymatous, but the developing asci destroy the pseudoparenchyma as they grow.

3. Paraphyses are present in the early stage of development, but have disappeared completely by the time the ascospores are mature.

4. The most important families are the Gnomoniaceae and the Diaporthaceae. The former is non-stromatic, the latter is stromatic. Many modern authors unite the two families under the name Diaporthaceae.

B. PROCEDURE:

Family: Gnomoniaceae

5. The Gnomoniaceae produce perithecia with well-developed, often long beaks. The bases of the perithecia are typically sunken in the substratum (not in a stroma) and the beaks protrude to the surface.

Genus: Gnomonia

6. Conidial stages: Most species of *Gnomonia* produce their conidia in acervuli. Make thin, free-hand sections through the acervuli of *Marssonina juglandis* (the imperfect stage of *Gnomonia leptostyla*). Note the two-celled sickle-shaped spores borne on short conidiophores, the latter arranged in bed-like masses. Other species of *Gnomonia* produce different types of conidia belonging to such form-genera as *Gloeosporium*, *Fusicoccum*, etc.

7. Some species of *Gnomonia* produce their conidia in pycnidia. Study a culture of *Gnomonia fragariae* which is producing pycnidia. The asexual stage is *Zythia fragariae*. Crush the pycnidium and observe the rather long, simple or branched conidiophores bearing conidia at the tips.

Family: Diaporthaceae

Genus: Diaporthe

8. The genus *Diaporthe* resembles *Gnomonia* except for the presence of a stroma in the former and the absence of one in the latter.

9. All species of Diaporthe appear to have imperfect stages which have the characteristics of the form-genus Phomopsis.

10. Study Phomopsis vexans, the universally distributed imperfect stage of Diaporthe vexans, on eggplant. Search for the long, curved stylospores interspersed with the oval pycnidiospores. Some strains do not produce stylospores, but only pycnidiospores.

11. Study the perithecia of some species of Diaporthe, such as Diaporthe phaseolorum. Note that the perithecial bases are sunken in the stroma and that the long perithecial necks protrude.

Genus: Glomerella

12. Make as complete a study of Glomerella cingulata as available material will permit.

    a. Somatic hyphae: Study several colonies of the fungus and note differences of color and aerial mycelium.

    b. Conidial stages: Observe apples which have been inoculated with Glomerella cingulata and kept in a moist chamber for a few days. Note the more or less concentric arrangement of the acervuli and the pink masses of conidia. Make a thin section through an acervulus and note how the conidia are borne. This stage of the fungus represents the form-genus Gloeosporium. Under certain conditions and on certain hosts (i. e. , Citrus) the acervuli bear long, bristle-like setae. Such setae are characteristic of the form-genus Colletotrichum (see question 6).

    c. Ascigerous stage: Observe the perithecia of Glomerella cingulata as they grow in culture. Crush a perithecium under a cover-glass and study the asci and the ascospores.

Genus: Endothia

13. Make as complete a study of Endothia parasitica as available material will permit.

    a. Somatic hyphae: Study the mycelium of the fungus from culture. Note the color of the colony; mount some hyphae and observe under the microscope.

    b. Conidial stage: Examine chestnut twigs and branches which show blight cankers. The pimply appearance of the canker is due to the numerous erumpent pycnidia. Spores are exuded in long cirri or "spore horns". Cut a thin longitudinal section through a pycnidium and mount in lactophenol. Note the labyrinthine internal structure and the enormous number of allantoid conidia. These are some of the characteristics of the form-genus Cytospora to which the imperfect stage belongs.

    c. Acigerous stage: Study longitudinal sections through a perithecial stroma. Note the flask-shaped perithecia with their long necks which reach the surface of the stroma. Observe the clavate asci and the two-celled ascospores.

14. Study other genera in this family as they are made available to you and learn to distinguish among them.

C. QUESTIONS:

1. Distinguish between the Diaporthales and the Sphaeriales.
2. What families are usually included in the Diaporthales?
3. What is meant by a perithecial centrum?
4. How important is centrum character in the classification of the Pyrenomycetes?
5. Discuss the genetic connections between perfect and imperfect stages in Gnomonia, Glomerella, and Diaporthe.
6. Discuss the validity of the form-genera Gloeosporium and Colletotrichum on the basis of known facts concerning variation in these fungi.
7. Discuss the importance of the Diaporthales from an economic standpoint.

D. TEXT REFERENCES:

Alexopoulos -- pp. 313-316.
Bessey -- pp. 280-283.

E. SELECTED REFERENCES:

1. BROOKS, F. T. 1910. The development of Gnomonia erythrostoma. Ann. Bot., 24:585-605.
2. COLE, J. R. 1935. Gnomonia nerviseda the perfect stage of the fungus that causes the vein spot disease of pecan foliage. Jr. Agr. Res., 50:91-96.
3. DRIVER, C. H. and H. E. WHEELER. 1955. A sexual hormone in Glomerella. Mycologia, 47:311-316.
4. EDGERTON, C. W. 1914. Plus and minus strains in the genus Glomerella. Am. Jr. Bot., 1:244-254.
5. HARTER, L. L. 1917. Pod blight of the lima bean caused by Diaporthe phaseolorum. Jr. Agr. Res., 11:473-504.
6. HUANG, SUNG. 1949. Morphological and cytological studies in Gnomonia fragariae Klebahn. M. S. Thesis, Michigan State College. 39 pp. 7 pls.
7. MC GAHEN, J. W. and H. E. WHEELER, 1951. Genetics of Glomerella. IX. Perithecial development and plasmogamy. Am. Jr. Bot., 38:610-617.
8. MILLER, J. H. 1928. Biologic studies in the Sphaeriales. Mycologia, 20:187-213, 305-339.
9. MILLER, J. H. 1949. A revision of the classification of the Ascomycetes with special emphasis on the Pyrenomycetes. Mycologia, 41:99-127.
10. MORGAN-JONES, J. F. 1953. Morpho-cytological studies of the genus Gnomonia. I. Svensk. Bot. Tidskr., 47:284-308.
11. MORGAN-JONES, J. F. 1958. Morpho-cytological studies of the genus Gnomonia. II. Svensk Bot. Tidskr., 52:363-372.
12. MORGAN-JONES, J. F. 1959. Morpho-cytological studies of the genus Gnomonia. III. Svensk Bot. Tidskr., 53: 81-101.
13. POMERLEAU, R. 1938. Recherches sur le Gnomonia ulmea (Schw.) Thum. Contr. de l'Inst. Bot. de l'Univ. de Montreal. No. 31, 139 pp. 3 figs. 30 pls.
14. SHEAR, C. L., N. E. STEVENS, and RUBY J. TILLER. 1917. Endothia parasitica and related species. U. S. Dept. Agr. Prof. Paper Ser. Bull., 380, 82 pp.
15. SHEAR, C. L. and ANNA K. WOOD. 1913. Studies of fungous parasites belonging to the genus Glomerella. U. S. Dept. Agr. Bur. Pl. Ind. Bull. 252. 105 pp. 4 figs. 18 pls.
16. TIMNICK, MARGARET BARTON, V. G. LILLY and H. L. BARNETT. 1951. Factors affecting sporulation of Diaporthe phaseolorum var. batatatis from soybean. Phytopath., 41:427-336.
17. WEHMEYER, L. E. 1926. A biologic and phylogenetic study of stromatic Sphaeriales. Am. Jr. Bot., 13:574-645.
18. WEHMEYER, L. E. 1933. The genus Diaporthe Nitschke and its segregates. Univ. of Michigan Press. Ann Arbor.
19. WELCH, A. W. and J. C. GILMAN. 1948. Hetero- and homothallic types of Diaporthe on soy beans. Phytopath., 38:628-637.
20. WHEELER, H. E., et al. 1948. Genetics of Glomerella. V. Crozier and ascus development. Am. Jr. Bot., 35:722-728.
21. WHEELER, H. E. 1954. Genetics and evolution of heterothallism in Glomerella. Phytopath., 44:342-345.
22. WHEELER, H. E. 1956. Linkage groups in Glomerella. Am. Jr. Bot., 43:1-6.
23. WHEELER, H. E. 1956. Sexual verses asexual reproduction in Glomerella. Mycologia, 48:349-353.
24. WHEELER, H. E., C. H. DRIVER and C. CAMPA. 1959. Cross- and self-fertilization in Glomerella. Am. Jr. Bot., 46:361-365.
25. WHEELER, H. E. and J. W. MC GAHEN. 1952. Genetics of Glomerella X. Genes affecting sexual reproduction. Am. Jr. Bot., 39:110-119.

F. GLOSSARY:

1. Acervulus (pl. acervuli; L. acervus = heap, dimin. form): a mat of hyphae giving rise to short conidiophores closely packed together forming a bed-like mass.
2. Cirrus (pl. cirri; L. cirrus = a curl): a curley, ribbon-like mass of spores issuing from a fruiting body, much like toothpaste from the opening of a tube.

3. Pycnidium (pl. pycnidia; Gr. pyknon = concentrated + idion, a dimin. suffix): an asexual, hollow fruiting body lined inside with conidiophores.
4. Seta (pl. setae; L. seta = bristle): a bristle-like hair.
5. Stylospore (Gr. stylos = column + sporos = seed, spore): an elongated, generally curved "spore" of unknown function produced by certain types of fungi.

Class Ascomycetes
Sub-Class Euascomycetidae
Series Pyrenomycetes

Order: Hypocreales

A. GENERAL REMARKS

1. The Hypocreales are typical Pyrenomycetes producing their asci in true perithecia. The perithecia may be borne on the mycelium without a stroma or they may be stromatic, sitting on top of the stroma or immersed within it.

2. The perithecia of the Hypocreales have a more or less soft, waxy or fleshy wall which is generally brightly colored (red, orange, blue, purple, etc.). These characteristics serve to distinguish this order from the Sphaeriales, but obviously there is no definite dividing line between the two groups.

3. In many species in which the development of the perithecium has been studied, it has been found that pseudoparaphyses (apical paraphyses according to Munk, 1954) are present in the ascocarp between the asci. This character may prove to be the most important distinguishing feature of the order Hypocreales.

4. The Hypocreales are divided into four families: the Nectriaceae, the Hypocreaceae, the Melanosporaceae and the Hypomycetaceae. The perithecia of the Nectriaceae are either non-stromatic, of if stromatic they are superficial on the stroma; those of the Hypocreaceae are immersed in the stroma. In the Melanosporaceae and the Hypomycetaceae no sterile threads have been found among the asci. The Melanosporaceae produce their perithecia free; the Hypomycetaceae produce them in a felt-like mass of hyphae, the subiculum.

B. PROCEDURE:

Family: Nectriaceae

Genera: Nectria, Gibberella

5. Make a study of *Nectria cinnabarina* in accordance with the following directions:

   a. Somatic hyphae: Examine a pure culture of *Nectria cinnabarina*. Note the color and the general characteristics of the colony. Mount a small portion of the colony and study the somatic hyphae.

   b. Conidial stage: Using a hand lens or a dissecting microscope, examine a *Nectria* canker with mature sporodochia. Note the color and shape of these structures. Cut a thin longitudinal section through a sporodochium and mount in water or lacto-phenol. Study the section under the microscope and observe the relationship of the conidiophores to the sporodochial tissues. Note the conidia and their method of attachment. The conidial stage of *Nectria cinnabarina* is *Tubercularia vulgaris*.

   c. Ascigerous stage: Using a dissecting microscope observe the clusters of dark red perithecia on the older stromata. Make a thin longitudinal section through a perithecium and mount. Find the asci and the two-celled, boat-shaped as-

cospores. Study stained sections of perithecia. Note the sterile threads which fill the perithecium. Miller (1949) calls these structures pseudoparaphyses.

6. Study Gibberella zeae, the cause of ear rot of corn. Pay particular attention to the conidial stage which belongs to the imperfect genus Fusarium. A large number of Hypocreales have a Fusarium imperfect stage.

Family: Hypocreaceae

Genera: Hypocrea, Chromocrea

7. Study any species of Hypocrea or Chromocrea that is available. Note that the perithecia are completely sunken in the stroma with only their ostioles protruding. Note the color of the stroma. Make a section through a stroma and observe the perithecial wall, the ostiole and the periphyses. Study the asci and the ascospores. Count the number of ascospores in a mature ascus. How are these ascospores formed?

Family: Melanosporaceae

Genus: Melanospora

8. Study a Petri dish culture of a species of Melanospora. Observe the perithecia under the high power of the stereomicroscope and note the ribbons of spores issuing from the perithecial ostioles. Mount a mature perithecium under a cover slip carefully so that you may be able to observe it intact. Note the transparent walls of the ascocarp. The asci are evanescent.

9. Study a young perithecium. Find the asci and ascospores.

Family: Hypomycetaceae

Genus: Hypomyces

10. Observe a mushroom that is parasitized by a species of Hypomyces. Note the felt-li' e subiculum of the parasite in which the reddish or orange perithecia are embedded. Carefully dig out a perithecium and mount in lacto-phenol. Crush the perithecium and observe the asci and the elongated ascospores.

C. QUESTIONS:

1. Characterize the Hypocreales as now delimited (Martin, 1961).
2. What are pseudoparaphyses? In what orders of fungi are they found?
3. Why does Munk (1957) call these structures apical paraphyses in the Nectriales? How does he say they differ from the pseudoparaphyses of other orders?
4. What is a sporodochium? In what form-family are sporodochial fungi placed?
5. Classify the imperfect stage of Nectria cinnabarina and Gibberella zeae, in the proper form-order, form-family, and form-genus.
6. Name some genera, other than the ones mentioned in this discussion, which belong to the Hypocreales.

D. TEXT REFERENCES:

Alexopoulos -- pp. 316-321.
Bessey -- 285-290.

F. SELECTED REFERENCES:

1. BLACKMAN, V. H. and E. J. WELSFORD. 1912. The development of the perithecium of Polystigma rubrum. Ann. Bot., 26:761-767.
2. BOOTH, C. and J. S. MURRAY. 1960. Calonectria hederae Arnaud and its Cylindrocladium conidial state. Trans. Br. Mycol. Soc., 43:69-72.
3. BUXTON, E. W. 1959. Production of a perfect stage in a nutritionally deficient mutant of pathogenic Fusarium oxysporum after ultra-violet irradiation. Nature (London), 184:1258.
4. CAYLEY, DOROTHY M. 1921. Some observations on the life history of Nectria galligena. Ann. Bot., 35:79-92.
5. CLEMENTS, F. E. and C. L. SHEAR. 1931. The genera of fungi. iv + 496 pp. 58 pls. H. W. Wilson Co., New York.
6. DOUGUET, G. 1955. Le genre Melanospora. Le Botaniste, 39:1-313.
7. EL-ANI, A. S. 1956. Cytogenetics of sex in Giberella cyanogena. Science, 123:850.
8. EL-ANI, A. S. 1959. Chromosome numbers in Hypocreales. I. Nuclear division in the ascus of Nectria pezzia. Am. Jr. Bot., 46:412-416.
9. GILLES, A. 1948. Evolution nucléaire et développement du perithèce chez Nectria flava. La Cellule 51:369-400.
10. GORDON, W. L. 1960. Is Nectria haematococca Berk. and Br. the perfect stage of Fusarium oxysporum Schl. forma pisi (Lindf.) S. and H.? Nature (London), 186:903.
11. HANLIN, R. T. 1961. Studies in the genus Nectria. I. Factors influencing perithecial formation in culture. Bull. Torrey Bot. Club, 88:95-103.
12. HAWKER, L. E. 1948. The effect of certain growth substances on the mycelial growth and fruiting of Melanospora destruens. Trans. Br. Mycol. Soc., 30:135-140.
13. HAWKER, L. E. 1948. Stimulation of the formation of perithecia of Melanospora destruens by small quantities of certain phosphoric esters of glucose and fructose. Ann. Bot., 12:77-79.
14. HIRSCH, HILDE E., W. C. SNYDER and H. N. HANSEN. 1949. Chromosome numbers in the Hypocreaceae. Mycologia, 41:411-415.
15. LINE, J. 1922. The parasitism of Nectria cinnabarina, (coral spot) with special reference to its action on red currant. Trans. Br. Mycol. Soc., 8:22-28.
16. LUTTRELL, E. S. 1944. The morphology of Sphaerostilbe aurantiicola (B. & Br.) Petch. Bull. Torrey Bot. Club, 71:599-619.
17. LUTTRELL, E. S. 1951. Taxonomy of the Pyrenomycetes. Univ. Missouri Studies 24, No. 3, 120 pp. Curators Univ. of Missouri, Columbia.
18. MATHIESON, M. J. 1952. Ascospore dimorphism and mating type in Chromocrea spinulosa (Fuckel) Petch n. comb. Ann. Bot. n.s., 64:449-466.
19. MILLER, J. H. 1949. A revision of the classification of the Ascomycetes with special emphasis on the Pyrenomycetes. Mycologia, 41:99-127.
20. MUNK, A. 1954a. An anatomic study of the genus Nectria, Fr. with remarks on the pattern of variation within the genus. 8th Int. Bot. Congr. Sect. 18, 19, 20:49-51.
21. MUNK, A. 1954b. The bases of the systematic relationships of the pyrenomycetes. 8th Int. Congr. Sect. 18, 19, 20:35-44.
22. MUNK, A. 1957. Danish pyrenomycetes. Dansk. Bot. Arkiv., 17:1-491.
23. RANZONI, F. V. 1956. The perfect stage of Flagellospora pencillioides. Am. Jr. Bot. 43:13-17.
24. SEAVER, F. J. 1910. Hypocreales. North American Flora, 3:1-56.
25. SEELER, E. V., JR. 1940. A monographic study of the genus Thyronectria. Jour. Arnold Arbor., 21:429-460.
26. SNYDER, W. C. and H. N. HANSEN. 1940. The species concept in Fusarium. Am. Jr. Bot., 27:64-67.

F. GLOSSARY:

1. Pseudoparaphyses (sing. pseudoparaphysis; Gr. pseudos = falsehood + paraphysis): sterile threads growing between the asci and attached both to the roof and to the base of an ascocarp. They are also known as paraphysoids.
2. Sporodochium (pl. sporodochia; Gr. sporos = seed, spore + docheion = container): a cushion-shaped stroma covered with conidiophores.
3. Subiculum (pl. subicula; L. subex = an underlayer): a felt-like mass of somatic hyphae resembling a stroma, on which or in which fruiting bodies are formed.

Class Ascomycetes
Sub-Class Euascomycetidae
Series Pyrenomycetes

Order Clavicipitales

A. GENERAL REMARKS:

1. The Clavicipitales are easily distinguished by the character of their asci and ascospores. The ascus has a thick cap, perforated by a central narrow canal through which the needle-shaped ascospores are successively ejected.

2. The Clavicipitales are parasitic on plants, insects, or on other fungi. There is only one family, the Clavicipitaceae.

Family: Clavicipitaceae

Genera: Claviceps, Cordyceps

3. Make a complete study of Claviceps purpurea in accordance with the following instructions:

   a. Somatic hyphae: If pure cultures of the organism are available study the characteristics of the colonies and of the somatic hyphae of which they are composed.

   b. Conidial stage: Study prepared slides showing sections of mycelial mats with layers of conidiophores. Observe the large numbers of conidia produced. The conidiiferous mycelial mats develop eventually into the sclerotia of the fungus.

   c. Sclerotial stage: Examine heads of rye infected with Claviceps purpurea. Note the size, shape, and color of the sclerotia. Study prepared slides of stained cross-sections of sclerotia. Note the pseudoparenchymatous tissue composing the rind and the interior of the sclerotium. Test the hardness of a sclerotium by cutting it with a scalpel.

   d. Ascigerous stage: Observe germinated sclerotia bearing stomata. Study stained longitudinal sections through a stroma. Note the position of the perithecia. Find the perithecial wall as distinguished from the stromatic tissue. Search your slide for a perithecium which has been sectioned exactly through the center; note the ostiole, the periphyses, the asci, and the ascospores.

4. Observe caterpillars parasitized by Cordyceps militaris. Examine the stromata of the fungus containing numerous perithecia. Make thin sections through a stroma or study stained sections and observe the perithecia, the asci, and the ascospores. Compare with Claviceps.

C. QUESTIONS:

1. Characterize the Clavicipitales.
2. Discuss the views of various authors (Stevens, 1913; Miller, 1949; Bessey, 1950; Luttrell, 1951, 1955; Gäumann, 1952; Martin, 1961) on the classification of these fungi.

3. In what form-genus is the imperfect stage of Claviceps purpurea classified?
4. What is a sclerotium? What is its function?
5. How are the ascospores of Claviceps purpurea released from the perithecia?
6. How does plasmogamy occur in Claviceps purpurea? What is the probable situation in Nectria galligena?
7. What is ergot? Ergotism? Ergotamine?
8. How are the sclerotia of Claviceps purpurea used medicinally?
9. What fungi are parasitized by the Clavicipitaceae?

D. TEXT REFERENCES:

Alexopoulos -- pp. 321-325.
Bessey -- pp. 287-289.

E. SELECTED REFERENCES:

1. CAREW, D. P. and A. E. SCHWARTING. 1959. The infection of rye callus with Claviceps purpurea. Am. Pharm. Assoc. J. Sci. Ed., 48:499-500.
2. DICKSON, J. G. 1947. Diseases of field crops. xii + 429 pp. 102 figs. McGraw-Hill Book Co., New York.
3. GÄUMANN, E. A. 1952. The fungi (Transl. by F. L. Wynd). 420 pp. 440 figs. Hafner Publishing Co., New York.
4. JENKINS, W. A. 1934. The development of Cordyceps agariciformis. Mycologia, 26:220-243.
5. JUNG, M. and H. ROCHELMEYER. 1960. Zur Morphologie und Cytologie von Claviceps purpurea (Tulasne) in saprophytischer Kultur. Beiter. Biol. Pflanzen., 35:343-378.
6. KILLIAN, K. 1919. Sur la sexualité de l'érgot du seigle, le Claviceps purpurea (Tulasne). Bull. Soc. Mycol. France, 35:182-197.
7. LUTTRELL, E. S. 1951. The taxonomy of the Pyrenomycetes. Univ. Mo. Stud. 24, No. 3. 120 pp. University of Missouri, Columbia.
8. LUTTRELL, E. S. 1955. The ascostromatic Ascomycetes. Mycologia, 47:511-532.
9. MAINS, E. B. 1957. Species of Cordyceps parasitic on Elaphomyces. Bull. Torrey Bot. Club, 83:243-251.
10. MARTIN, G. W. 1961. Key to the families of fungi. In Dictionary of the fungi, pp. 497-517. G. C. Ainsworth. Commonwealth Mycological Institute, Kew.
11. MILLER, J. H. 1949. A revision of the classification of the Ascomycetes with special emphasis on the Pyrenomycetes. Mycologia, 41:99-127.
12. STEVENS, F. L. 1913. The fungi which cause plant disease. ix + 754 pp. 449 figs. The Macmillan Co., New York.
13. VINING, L. C. and W. A. TABER. 1959. Estimation of ergot alkaloids in cultures of Claviceps purpurea. Can. Jr. Microbiol., 5:441-451.

F. GLOSSARY:

1. Sclerotium (pl. sclerotia; Gr. skleron = hard): a hard resting body, resistant to unfavorable conditions, which may remain dormant for long periods of time and germinate upon the return of favorable conditions.

Class Ascomycetes – Sub-Class Euascomycetidae – Series Discomycetes

A. GENERAL REMARKS:

1. The Discomycetes bear their asci in open ascocarps of various types, all referred to under the general term apothecium. The apothecia are, most often, disk-shaped or cup-shaped, but are sometimes considerably modified into tongue-like, spongy, convoluted, or saddle-shaped, pileate structures, Apothecia may be stipitate or sessile.

2. In most Discomycetes the asci are unitunicate. Some of the discomycetous lichen fungi, however, bear their ascospores in bitunicate asci.

3. The epigean, non-lichen-forming Discomycetes fall into two general groups on the basis of ascus dehiscence. The first group (inoperculate), in which the asci burst irregularly at the tips to release the spores, includes two orders, the Ostropales and the Helotiales; the second group (operculate and sub-operculate), in which the asci are equipped with a hinged operculum or a plug, or open by a regular, longitudinal slit, includes the order Pezizales.

4. The hypogean Discomycetes consist of the order Tuberales, commonly called truffles.

Inoperculate Discomycetes

Order: Ostropales

5. The order Ostropales is a rather small group of epigean, inoperculate Discomycetes which produce thread-like (filiform) ascospores. They are not very common and will not be discussed here.

Order: Helotiales

A. GENERAL REMARKS:

6. The order Helotiales is the largest and, from the economic standpoint, the most important of all the discomycetous orders. It is characterized by its fleshy apothecia which bear elongated asci with oval, spherical, or elongated ascospores.

7. The number of families included in this order varies according to the author. We shall discuss representatives of four of the six or seven families generally placed in this order.

B. PROCEDURE:

Family: Sclerotiniaceae
Genera: Monilinia, Sclerotinia

8. Make a complete study of Monilinia fructicola.

a. Somatic hyphae: If fresh peaches are available, mount a small portion of tissue infected with the fungus. Note the ease with which the host cells can be separated. Observe the numerous hyphae of the parasite. If fruit is not available make a study of the hyphae from culture.

b. Conidial stage: Scrape some of the gray powdery substance from the surface of an infected peach or peach mummy, and mount. Note the branched conidiophores and the characteristic chains of lemon-shaped conidia. Study the way in which conidia are formed.

c. Spermatial stage: Examine a culture of Monilinia fructicola which is producing spermatia (microconidia). Mount a small portion of the mycelium and search for spermatiophores and spermatia. Note the rozette-like aggregations of the spermatiophores on the hyphae, and the chains of spermatia.

d. Ascigerous stage: Examine peach mummies which have overwintered on the ground and bear apothecia. Make a thin section of a portion of an apothecium to show the hymenial layer. Mount in 1% iodine solution and observe the asci with ascospores and the numerous paraphyses.

9. In the same way study the somatic hyphae, sclerotia, spermatia, and apothecia of Sclerotinia sclerotiorum.

Family: Dermataceae

Genera: Higginsia, Diplocarpon

10. Make a study of the various stages of Higginsia hiemalis, the cause of cherry leaf spot.

a. Somatic hyphae: If cultures of this organism are available, study the somatic hyphae growing in agar.

b. Conidial stage: Make a free hand section of a cherry leaf collected in late summer, through an acervulus of Higginsia hiemalis and find the long, septate conidia. These belong to the form-genus Cylindrosporium.

c. Spermatial stage: Spermatiophores and spermatia (microconidia) have been reported as occurring in the conidial acervuli. Search your sections for such structures.

d. Ascigerous stage: Examine overwintered leaves for apothecia. Study the ascocarps, asci, ascospores, and paraphyses.

11. Diplocarpon rosae and other species.

a. Study the conidial and ascigerous stages of Diplocarpon rosae, the cause of black spot of roses, by making thin cross-sections through an acervulus and an apothecium. The conidial stage of this organism exhibits the characteristics of the form-genus Marssonina, and belongs to the species Marssonina rosae. Diplocarpon earliana, the cause of leaf scorch of strawberry also has a Marssonina imperfect stage (Marssonina fragariae).

b. If material is available, study Diplocarpon soraueri (Entomosporium maculatum), the cause of black spot of quince. Note the peculiar conidia which bear hyaline setae and resemble insects. This fungus also attacks certain varieties of pear.

Family: Phacidiaceae

Genus: Rhytisma

12. Examine young stromata of Rhytisma acerinum on maple leaves. Note the radiate lines so characteristic of these stromata. Section young stromata and search for the conidial (spermatial?) stage of the fungus. These "conidia" are borne in acervulus-like structures and do not germinate. Their function is unknown.

13. Examine overwintered stromata which have been kept between wet newspapers for 48 hours or longer. As the moist paper is lifted, watch carefully for clouds of ascospores which should puff out of the asci. Note the wide, radiate fissures on the stroma.

14. Make a thin section through the stroma and mount; study the asci and the filiform ascospores.

15. Study stained sections through a stroma. Locate the apothecia as distinguished from the stromatic tissue.

Family: Geoglossaceae

Genera: Geoglossum, Spathularia, Trichoglossum

16. Examine representatives of as many genera of the Geoglossaceae as are available and study the ascocarps, asci, ascospores, and paraphyses. No conidial stages are known in this family.

C. QUESTIONS:

1. Characterize the Helotiales.
2. Construct a dichotomous key to the families in this order which you have studied.
3. How does an apothecium differ from a perithecium?
4. What is a peach mummy? What part does it play in the life cycle of Monilinia fructicola? Distinguish between mummies that cling to the trees and mummies that overwinter on the ground from the standpoint of primary infection in the spring.
5. What is the probable function of the spermatia of Monilinia fructicola? Give reasons for your answer.
6. Where and under what circumstances do the apothecia of Monilinia fructicola originate?
7. How does Monilinia differ from Sclerotinia?
8. How are the spermatia of Higginsia hiemalis produced? How do they appear to function?
9. Describe the life history of Rhytisma acerinum based on the findings of Jones.
10. Discuss the various views on the classification and relationships of the Phacidiaceae.

D. TEXT REFERENCES:

Alexopoulos -- pp. 333-343.
Bessey -- pp. 230-236.

E. SELECTED REFERENCES:

1. ATKINSON, G. F. 1909. The perfect stage of leaf spot of pear and quince. Sci., n.s., 30:452.
2. BACHE-WIIG, SARA. 1940. Contributions to the life history of a systemic parasite Cryptomycina pteridis. Mycologia, 32:214-250.

3. BACKUS, M.P. 1934. Initiation of the ascocarp and associated phenomena in Coccomyces hiemalis. Contrib. Boyce Thompson Inst., 6:339-379.
4. BATRA, L. R. 1960. The species of Ciborinia pathogenic to Salix, Magnolia and Quercus. Am. Jr. Bot., 47:819-827.
5. BUCHWALD, N. F. 1956. On the dimorphism of the ascospores and their arrangement in the ascus of Monilinia oxycocci (Wor.) Honey (syn. Sclerotinia oxycocci Wor.) Friesia, 5:196-203.
6. CHADEFAUD, M. 1960. Traité de botanique systématique. Vol. I. xv + 1018 pp. 713 figs.
7. DARKER, G. D. 1932. The Hypodermataceae of conifers. Contribs. Arnold Arboretum, Harvard Univ., 1:1-132. 27 pls.
8. DENNIS, R. W. G. 1956. A revision of the British Helotiaceae in the herbarium of the Royal Botanic Gardens, Kew, with notes on related European species. Commonw. Myc. Inst. Myc. Papers, 62:1-216.
9. DENNIS, R. W. G. 1960. British cup fungi and their allies. xxiv + 280 pp. 20 figs. 40 col. pls.
10. DRAYTON, F. L. 1932. The sexual function of the microconidia in certain Discomycetes. Mycologia, 24:345-348.
11. DRAYTON, F. L. 1934. The sexual mechanism of Sclerotinia gladioli. Mycologia, 26:46-72.
12. DRAYTON, F. L., and J. W. GROVES. 1952. Stromatinia Narcissi, a new dimorphic discomycete. Mycologia, 44:119-140.
13. DUFF, G. H. 1922. Development in the Geoglossaceae. Bot. Gaz., 74:264-291.
14. DURAND, E. J. 1908. A monograph of the Geoglossaceae. Ann. Mycol., 6:387-477.
15. DURAND, E. J. 1921. New or noteworthy Geoglossaceae. Mycologia, 13:184-187.
16. GROVES, J. W. and C. A. LOVELAND. 1953. The connection between Botryotinia fuckeliana and Botrytis cinerea. Mycologia, 45:415-425.
17. HASKINS, R. H. 1960. A heterothallic inoperculate Discomycete: morphology and development. Can. Jr. Microbiol., 6:225-231.
18. HEUBERGER, J. W. 1934. Fruit-rotting Sclerotinias IV. A cytological study of Sclerotinia fructicola (Wint.) Rehm. Maryland Agr. Exp. Sta. Bull., 371:167-190.
19. HIGGINS, B. B. 1914. Contribution to the life history and physiology of Cylindrosporium on stone fruits. Am. Jour. Bot., 1:145-173.
20. HONEY, E. E. 1928. The monilioid species of Sclerotinia. Mycologia, 20:127-157.
21. HONEY, E. E. 1936. North American species of Monilinia. I. Occurrence, grouping and life history. Am. Jr. Bot., 23:100-106.
22. IMAI, S. 1941. Contributiones ad studia monographica Geoglossacearum. III. Studia classificationis phylogenicarum. Sci. Rep. Yokohama Nat. Univ. II., 5:1-8.
23. JONES, S. G. 1925. Life history and cytology of Rhytisma acerinum (Pers.). Fr. Ann. Bot., 39:41-75.
24. KOBAYASI, Y. 1937. On the gelatinous cup fungi, Bulgaria group. Jr. Jap. Bot., 13:510-520.
25. KORF, R. P. 1951. A monograph of the Arachnopezizae. Lloydia, 14:129-180.
26. KORF, R. P. 1957a. Nomenclatural notes. II. On Bulgaria, Phaeobulgaria and Sarcosoma. Mycologia, 49:102-106.
27. KORF, R. P. 1958. Japanese Discomycetes. Notes I-VIII. Sci. Reports Yokohama Nat. Univ. Sec. II. No. 7:7-35.
28. LE GAL, M. 1953. Les discomycètes de Madagascar. 465 pp. 172 figs. Mus. Nat. Hist. Nat. Paris.
29. MAINS, E. B. 1954. North American species of Geoglossum and Trichoglossum. Mycologia, 46:586-631.
30. MAINS, E. B. 1955. North American hyaline-spored species of the Geoglossaceae. Mycologia, 47:846-877.
31. MAINS, E. B. 1956a. The relationship of Cudoniella and Helotium. Mycologia, 48:410-419.
32. MAINS, E. B. 1956b. North American species of the Geoglossaceae. Tribe Cudonieae. Mycologia, 48:694-710.
33. MARTIN, G. W. 1961. Key to the families of fungi. In Ainsworth's Dictionary of the fungi. pp. 497-517. Com. Mycol. Inst., Kew.
34. NANNFELDT, J. A. 1932. Studien über die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. Nova Acta Regiae Soc. Sci. Upsalensis, Ser. IV, 8:1-368.
35. RAMAMURTHI, C. S., R. P. KORF and L. R. BATRA. 1957. A revision of the North American species of Chlorociboria (Sclerotiniaceae). Mycologia, 49:854-863.
36. SANTESSON, R. 1945. Cyttaria, a genus of inoperculate discomycetes. Svensk. Bot. Tidskr., 39:319-345.
37. SEAVER, F. J. 1951. The North American cup fungi (Inoperculates). ix + 428 pp. Pls. 76-150. Frontis. (col.). Published by the author, New York.
38. THIND, K. S. and G. W. KEITT. 1949. Studies on variability of Sclerotinia fructicola (Wint.) Rehm. Phytopath., 39:621-636.
39. WHETZEL, H. H. 1943. The spermodochidium, an unusual type of spermatial fruit-body in the Ascomycetes. Mycologia, 35:335-338.
40. WHITE, N. H. 1954. The development of the ascocarp of Cyttaria gunnii Berk. Trans. Br. Mycol. Soc., 37:431-436.
41. WILSON, J. M. 1937. The structure of galls formed by Cyttaria septentrionalis on Fagus Moorei. Proc. Linn. Soc. N.S.W., 62:1-8.
42. WILSON, M., MARY NOBLE, and ELIZABETH GRAY. 1954. Gloeotinia--a new genus of the Sclerotiniaceae. Trans. Br. Mycol. Soc., 37:29-32.

F. GLOSSARY:

1. Epigean (Gr. epi = upon + ge = earth): above the ground.
2. Hypogean (Gr. hypo = under + ge = earth): below the ground.
3. Inoperculate (L. in = without + operculum = lid): without an operculum.
4. Operculate (L. operculum = lid): with an operculum.
5. Spermatium (pl. spermatia; Gr. spermation = little seed): a non-motile, uninucleate, spore-like, male structure which empties its contents into a receptive female structure during plasmogamy.
6. Sub-operculate (L. sub = under, less than - operculum = lid): with a plug like structure in a thickened, apical, ascal ring.

Class Ascomycetes – Sub-Class Euascomycetidae – Series Discomycetes

Operculate and Sub-operculate Discomycetes

Order: Pezizales

A. GENERAL REMARKS:

1. The Pezizales include a large number of organisms most of which are saprobic, but some of which appear to form mycorrhizae.

2. Three families are recognized here: Sarcoscyphaceae, Pezizaceae, and Helvellaceae. Other authors divide the Pezizales into as many as seven families.

B. PROCEDURE:

Family: Sarcoscyphaceae

3. The asci in this family are long, slender, and sub-operculate. The ascal opening is typically oriented obliquely. There is a thickened apical ring with a plug or hinged operculum. The ascospores are always one-celled.

Genera: Sarcoscypha, Urnula

4. If freshly collected ascocarps of *Sarcoscypha coccinea* or *Urnula craterium* are available, place them in a moist chamber and store in the refrigerator at the beginning of the laboratory period. Toward the end of the period remove them from the refrigerator and open the lid of the moist chamber. Observe the cloud of ascospores puffed from the apothecia.

5. Catch some of the ascospores as they are puffed, on the surface of corn meal agar in a Petri dish. Study the type of germination in the two species. Compare.

Family: Pezizaceae:

6. The fungi we place in this family produce cup- or disk-shaped apothecia which are not clearly differentiated into stalk and pileus. The asci are operculate.

Genera: Ascobolus, Ascodesmis, Saccobolus

7. These are mostly coprophilous fungi which are often segregated into a separate family, Ascobolaceae. Examine dung which has been kept in a moist chamber and has developed ascocarps of these fungi. Study the diminutive apothecia, the exerted asci, and the purplish ascospores. Note the spore clusters of *Saccobolus*.

Genera: Anthracobia, Peziza, Pyronema, Scutellinia

8. Study representatives of as many of the genera listed as are made available to you. Conidial stages of some species such as Peziza repanda and Peziza vesiculosa have been produced in culture. If fresh apothecia of either species are available germinate some ascospores on agar by inverting a plate over a puffing apothecium and study the germination of the spores, the hyphae, and the conidia produced in culture.

Family: Helvellaceae

Genera: Helvella, Gyromitra, Morchella, Verpa

9. In the Helvellaceae the ascocarps are pileate being distinctly differentiated into stalk and pileus. The genera listed above are sometimes separated into two families. Helvella and Gyromitra, together with other genera, are placed in the Helvellaceae whereas Morchella and Verpa are placed with others in the Morchellaceae.

10. As treated here, the family includes the morels (Morchella), the bell morels (Verpa), the false morels (Gyromitra), and the saddle fungi (Helvella). Study the various types of apothecia and learn to recognize the four genera. Learn to distinguish the edible types from the poisonous.

11. Mount a portion of the hymenial layer of some of these forms and study the asci, ascospores and paraphyses.

C. QUESTIONS:

1. Characterize the Pezizales.
2. Discuss sexual reproduction in Ascobolus.
3. What are the three prominent theories concerning ascus development in Pyronema omphalodes? Which of these is now generally accepted as representing the true situation in the Ascomycetes.
4. What is mycorrhiza?
5. How do the true morels differ from the false morels, the bell morels, and the saddle fungi?
6. What is the poisonous principle found in the Helvellaceae?
7. What technique is used to induce germination of the ascospores of Ascobolus and its relatives?
8. Discuss the classification of the Pezizales, contrasting the conservative view as exemplified by Bessey (1950) and Martin (1961) with the newer classification proposed by Le Gal (1947, 1953) and by Dennis (1960).

D. TEXT REFERENCES:

Alexopoulos -- pp. 345-354.
Bessey -- pp. 227-229.

E. SELECTED REFERENCES:

1. ALEXOPOULOS, CONST. J. and E. E. BUTLER. 1949. Conidia-like structures in Plectania coccinea. Mycologia, 41:180-182.
2. BISTIS, G. 1956. Studies on the genetics of Ascobolus stercorarius (Bull.) Schrot. Bull. Torrey Bot. Club, 83:35-61.
3. BISTIS, G. 1956. Sexuality in Ascobolus stercorarius. I. Morphology of the ascogonium; plasmogamy; evidence for a sexual hormonal mechanism. Am. Jr. Bot., 43:389-394.

4. BISTIS, G. 1957. Sexuality in Ascobolus stercorarius. II. Preliminary experiments on various aspects of the sexual process. Am. Jr. Bot., 44:436-443.
5. BROCK, T. D. 1951. Studies on the nutrition of Morchella esculenta Fries. Mycologia, 43:402-422.
6. BULLER, A. H. R. 1934. Researches on fungi. Vol. VI. xii + 513 pp. 231 figs. Longmans, Green & Co., London.
7. CAIN, R. F. and NANCY A. HASTINGS. 1956. Studies of soil fungi. II. A new species of Sphaerospora with a Botrytis-like stage. Can. Jr. Bot., 34:360-376.
8. CHADEFAUD, M. 1946. Les asques para-operculés et la position systématique de la Pezize Sarcoscypha coccinea Fries ex Jacquin. Compt. Rend., 222:753-755.
9. DAVIDSON, R. W. 1950. Urnula craterium is possibly the perfect stage of Strumella coryneoidea. Mycologia, 42:735-742.
10. DENIS, A. 1961. Une intoxication collective par Gyromitra esculenta en Normandie. Bull. Soc. Mycol France, 77:64-67.
11. DENISON, W. C. 1959. Some species of the genus Scutellinia. Mycologia, 51:605-635.
12. DENNIS, R. W. G. 1960. British cup fungi and their allies. xxiv + 280 pp. 20 figs. 40 col. pls.
13. DODGE, B. O. 1912. Methods of culture and the morphology of the archicarp in certain species of the Ascobolaceae. Bull. Torrey Bot. Club, 39:139-197.
14. DOWDING, E. S. 1931. The sexuality of Ascobolus stercorarius and the transportation of the oidia by mites and flies. Ann. Bot., 45:621-637.
15. GAMUNDI, IRMA J. 1956. El Genero Scutellinia en la Argentina. Contribuciones Cientificas, Serie Botanica Vol. I., No. 2.
16. GAMUNDI, IRMA J. 1959. Addenda a las especies Argentinas de Cookeina kuntze. Boletin de la Soc. Argentina de Bot., 7:201-204.
17. GAMUNDI, IRMA J. 1960. Discomycetes operculados de la Argentina. Familias: Pezizaceae y Humariaceae. Lilloa, 30:257-338.
18. GWYNNE-VAUGHAN, H. C. I. 1937. Contributions to the study of Lachnea melaloma. Ann. Bot., n.s., 1:99-105.
19. GWYNNE-VAUGHAN, H. C. I. and H. S. WILLIAMSON. 1927. Germination in Lachnea cretea. Ann. Bot., 41:489-495.
20. GWYNNE-VAUGHAN, H. C. I. and H. S. WILLIAMSON. 1931. Contribution to the study of Pyronema confluens. Ann. Bot., 45:355-371.
21. GWYNNE-VAUGHAN, H. C. I. and H. S. WILLIAMSON. 1932. The cytology and development of Ascobolus magnificus. Ann. Bot., 46:653-670.
22. HARPER, R. A. 1900. Sexual reproduction in Pyronema confluens and the morphology of the ascocarp. Ann. Bot., 14:321-400.
23. HEIM, R. 1936. La culture des morilles. Rev. Mycol. (Suppl.), 1:10-11, 19-25. Illustr.
24. INGOLD, C. T. 1954. Aquatic Ascomycetes: Discomycetes from lakes. Trans. Br. Mycol. Soc., 37:1-18.
25. INGOLD, C. T. 1956. Dispersal in cup fungi. Essex Naturalist 29, Part 5.
26. KANOUSE, BESSIE B. 1948. The genus Plectania and its segregates in North America. Mycologia, 40:482-497.
27. KORF, R. P. 1952. Two techniques for showing spore markings in operculate Discomycetes. Trans. Brit. Mycol. Soc., 35:24-25.
28. KORF, R. P. 1954. A revision of the classification of operculate Discomycetes (Pezizales). Rapp. Comm. VIII Congr. Int. Bot. I. 18-20:80.
29. KORF, R. P. 1960. Jafnea, a new genus of the Pezizaceae. Nagaoa, 7:3-8.
30. LE GAL, M. 1947. Recherches sur les ornamentations sporales des discomycètes operculés. Ann. Sci. Nat. Bot. xi, 8:73-297.
31. Le GAL, M. 1953. Les discomycètes de Madagascar. 465 pp. 172 figs. Mus. Nat. Hist. Nat. Paris.
32. LE GAL, M. 1960. Les discomycètes de l'herbier Crouan. Ann. Sci. Nat. Bot. Biol. Veg. Ser. 12, 1:441-467.
33. MC CUBBIN, W. A. 1910. Development of the Helvellinaceae. I. Helvella elastica. Bot. Gaz., 49:195-206.
34. NANNFELDT, J. A. 1937. Contribution to the mycoflora of Sweden. 4. On some species of Helvella, together with a discussion of the natural affinities within Helvellaceae and Pezizaceae, tribe Acetabulae. Svensk Bot. Tidsk., 31:47-66.
35. NANNFELDT, J. A. 1949. Contributions to the mycoflora of Sweden. 7. A new winter discomycete, Urnula hiemalis Nannf. n. sp. and a short account of the Swedish species of the Swedish species of the Sarcoscyphaceae. Svensk. Bot. Tidsk., 43:468-484.
36. NUSSLE, HELENE A. 1936. The genus Underwoodia. Mycologia, 28:236-240.
37. OLIVE, L. S. 1950. A cytological study of ascus development in Patella melaloma (Alb. & Schw.) Seaver. Am. Jr. Bot., 37:757-762.
38. ROBBINS, W. J. and ANNETTE HERVEY. 1959. Wood extract and growth of Morchella. Mycologia, 51:356-363.
39. ROSINSKI, M. A. 1953. Two types of spore germination in Sarcoscypha coccinea (Scop ex Fr.) Lambotte. Mycologia, 45:302-306.
40. ROSINSKI, M. A. 1956. Development of the ascocarp of Anthracobia melaloma. Mycologia, 48:506-533.
41. SEAVER, F. J. 1928. The North American cup-fungi (Operculates). 284 pp. 5 figs. 45 pls. Frontis. (col.). Published by the author, New York. Supplement. 1942. 1-viii + 285-377. Pls. 46-74.
42. SMITH, A. H. 1949. Mushrooms in their natural habitats. Vol. I. Text, xiv + 626 pp. 11 figs. Vol. II. Illustration. 231 stereo-color transparencies in 33 reels for Viewmaster. Sawyer's Inc., Portland, Oregon.
43. SMITH, A. H. 1958. The mushroom hunter's field guide. 195 pp. Illustr. Univ. of Michigan Press, Ann Arbor.
44. THIND, K. S., EDITH K. CASH, and P. SINGH. 1959. The Pezizaceae of the Mussoorie Hills (India). VII. Mycologia, 51:457-464.
45. WILSON, IRENE M. 1952. The ascogenous hyphae of Pyronema confluens. Annals of Botany, N.S. 16:321-339.
46. WOLF, F. A. 1958. Mechanism of apothecial opening and ascospore expulsion by the cup-fungus Urnula craterium. Mycologia, 50:837-843.
47. WOOD, J. L. 1953. A cytological study of ascus development in Ascobolus magnificus Dodge. Bull. Torrey Bot. Club, 80:1-15.
48. YU CHUAN-CHANG, CLARE. 1954. The culture and spore germination of Ascobolus with emphasis on A. magnificus. Am. Jr. Bot., 41:21-30.

F. GLOSSARY:

1. Mycorrhiza (pl. mycorrhizae; Gr. mykes = mushroom, fungus + rhiza = root): a root infected with a mildly parasitic fungus said to actually benefit the root by supplying nutrients from the soil which are otherwise unavailable to the root.
2. Pileate (L. pileus = cap): a sporophore which is differentiated into a stalk and cap.
3. Pileus (pl. pilei; L. pileus = cap): upper portion or cap of certain types of ascocarps and basidiocarps.

Class Ascomycetes – Sub-Class Euascomycetidae – Series Discomycetes

Hypogean Discomycetes

Order: Tuberales

A. GENERAL REMARKS:

1. The Tuberales are hypogean Discomycetes with closed or nearly so, globose ascocarps bearing the asci along the walls of internal chambers. They are commonly called truffles and some species are considered to be the most delectable of fungi.

2. The order is subdivided into three families: the Geneaceae, the Tuberaceae, and the Terfeziaceae.

B. PROCEDURE:

3. Examine ascocarps representative of each family. Note that the chambers in some forms open to the outside whereas in others the ascocarps are completely closed.

4. Study stained slides with longitudinal sections of the ascocarps of *Tuber*. Observe the internal convolutions which form the walls of the chambers; study the asci and the ascospores.

C. QUESTIONS:

1. Give reasons for classifying the Tuberales with the Discomycetes.
2. In what group of Ascomycetes were the Terfeziaceae formerly classified? Why?

D. TEXT REFERENCES:

Alexopoulos -- pp. 354-355.
Bessey -- pp. 236-239.

E. SELECTED REFERENCES:

1. CHRISTENSEN, C. M. 1961. The molds and man. Ed. 2. ix + 238 pp. 7 figs., 14 pls. University of Minnesota Press, Minneapolis.
2. GILKEY, HELEN M. 1916. A revision of the Tuberales of California. University of California Publ. Bot., 6:275-356.
3. GILKEY, HELEN M. 1939. Tuberales of North America. Oregon State Monographs, 1, 63 pp.
4. GILKEY, HELEN M. 1954a. Tuberales. North American Flora, II, 1:1-36.
5. GILKEY, HELEN M. 1954b. Taxonomic notes on the Tuberales. Mycologia, 46:783-793.
6. PARKS, H. F. 1920. California hypogeous fungi -- Tuberaceae. Mycologia, 13:301-314.

Class Ascomycetes– Sub-Class Loculoascomycetidae

A. GENERAL REMARKS:

1. The Loculoascomycetidae produce bitunicate asci in ascostromata. The ascocarp centrum may be devoid of sterile threads or may contain pseudoparaphyses. The ascocarp opens by a lysigenous pore formed above the asci. Periphyses may be present but are characteristically absent.

2. The ascostroma may contain many locules (pluriloculate) or only a single locule (uniloculate). In the latter instance it resembles a perithecium and is difficult to distinguis from it. Such a uniloculate, perithecium-like ascostroma is called a pseudothecium.

3. The Loculoascomycetidae are classified into five orders: Myriangiales, Dothideales, Pleosporales, Hysteriales, and Microthyriales. We shall study only three of these.

Order: Dothideales

4. The Dothideales form their bitunicate asci singly, in fascicles, or in a layer within the ascostroma. As the asci grow, they push through the stromatic tissue and dissolve a cavity (locule) around them. There are no interascal threads of any sort, but remnants of the stroma between the asci, resembling pseudoparaphyses, are found in some forms.

5. The Dothideales include four families: Dothideaceae, Dothioraceae, Pseudosphaeriaceae, and Capnodiaceae. The inclusion of the last of these is tentative.

B. PROCEDURE:

Family: Dothideaceae

6. The asci, in this family, are formed in fascicles in a uniloculate or pluriloculate ascostroma.

Genus: Mycosphaerella

7. Study several species of Mycosphaerella and note the two-celled, hyaline ascospores. Imperfect stages vary in different species; they are distributed among the form-genera Phyllosticta, Ascochyta, Septoria, Ramularia, Cercospora, etc.

Genus: Guignardia

8. Make as complete a study of Guignardia bidwellii as available material permits. Guignardia differs from Mycosphaerella in that its ascospores are one-celled or very unequally two-celled.

   a. Somatic hyphae: Examine a pure culture of Guignardia bidwellii. Note the characteristic color of the colony. Mount a portion of the mycelium and study the various types of hyphae you will find.

   b. Conidial stage: Examine leaves of grape (Vitis), Boston ivy or Virginia creeper (Parthenocissus) infected with this fungus. Note the characteristic

necrotic spots on the leaves bearing the pycnidia of the fungus arranged in a circle. Make a section through a pycnidium, or dig an entire pycnidium out of the leaf, and mount in water or lacto-phenol, depending as to whether the material you are using is fresh or dried. Note the position of the pycnidium with reference to the leaf tissues. Study the shape and contents of the pycnidiospores (conidia). The conidial stage is Phyllosticta labruscae.

c. Spermogonial stage: Search infected berries for the spermogonia of the fungus. These resemble pycnidia, but contain enormous numbers of minute bacillary structures (spermatia or microconidia) which do not germinate and whose function has not been established. They are believed to function in spermatization.

d. Ascigerous stage: Examine grape berries bearing the ascocarps of this fungus. Crush an ascocarp under a cover glass and study the ascospores most of which are unequally two-celled. The short cell, (sometimes absent) is sterile; it does not germinate. Note that some of the bodies which are hardly distinguishable from ascocarps bear nothing but an abundance of oil droplets. These have been called the pycnosclerotia. They are probably immature ascocarps or pycnidia.

Family: Capnodiaceae

9. The stroma of the Capnodiaceae is carbonaceous and often extensively branched. The dark colored mycelium is superficial and is usually associated with insect secretions on living plants.

10. Study any of the Capnodiaceae which may be growing on plants in the greenhouse, such as Capnodium, Scorias, Limacinia, etc. Examine the superficial hyphae. Mount an ascocarp in lacto-phenol, crush it gently under a cover slip and force out the asci and ascospores. Note the color, shape, and size of the ascospores.

11. Study the pycnidia of the Capnodiaceae. Note how they differ from the ascostromata.

C. QUESTIONS:

1. Characterize the Loculoascomycetidae.
2. How does a bitunicate ascus differ from an unitunicate ascus?
3. How does a pseudothecium differ from a perithecium?
4. Construct a key to the orders of the Loculoascomycetidae.
5. Define the Order Dothideales.
6. Describe the formation of spermatia in Mycosphaerella. Describe the formation of ascocarps.
7. Of what phylogenetic significance are the spermogonia of Mycosphaerella said to be? (Consult Bessey, 1950)
8. List some important plant diseases caused by members of the Dothideales and name the causal organism for each.
9. What are the Capnodiaceae? What are they commonly called?
10. Why is the taxonomic position of the Capnodiaceae highly controversial?

D. TEXT REFERENCES:

Alexopoulos -- pp. 364-366, 370-377.
Bessey -- pp. 290-293; 296-299.

E. SELECTED REFERENCES:

1. BARR, M. E. 1958. Life history studies on Mycosphaerella tassiana and M. typhae. Mycologia, 50:501-513.
2. CALPOUZOS, L. 1955. Studies on the Sigatoka disease of bananas and its fungus pathogen. ix + 70 pp. Illus. Atkins Garden and Research Laboratory, Cienfuegos, Cuba.
3. CALTRIDER, P. G. 1961. Growth and sporulation of Guignardia bidwellii. Phytopath., 51:860-863.
4. CHADEFAUD, M. 1954. Sur les asques de deux Dothidéales. Bull. Soc. Mycol. France, 70:99-108.
5. FRASER, LILIAN. 1935. An investigation of the sooty moulds of New South Wales. II. The life histories and systematic positions of Aithaloderma and Capnodium, together with descriptions of new species. Proc. Linn. Soc. N. S. Wales, 60:97-118.
6. FRASER, LILIAN. 1937. The distribution of sooty-mould fungi and its relation to certain aspects of their physiology. Proc. Linn. Soc. N. S. Wales, 62:25-56.
7. GÄUMANN, E. A. 1949. The Fungi (Transl. by F. L. Wynd). 420 pp., 440 figs. Hafner Publishing Co., New York.
8. HIGGINS, B. B. 1920. Morphology and life history of some Ascomycetes with special reference to the presence and function of spermatia. I. Am. Jour. Bot., 7:435-444.
9. HIGGINS, B. B. 1929. Morphology and life history of some Ascomycetes with special reference to the presence and function of spermatia. II. Am. Jour. Bot., 16:287-296.
10. HIGGINS, B. B. 1936. Morphology and life cycle of some Ascomycetes with special reference to the presence and function of spermatia. III. Am. Jour. Bot., 23: 598-602.
11. LUC, M. 1952. Structure et développement de deux Dothideales: Systremma natans (Tode) The. et Syd. et Bertia moriformis (Tode) de Not. Bull Soc. Mycol. France, 68:149-164.
12. LUTTRELL, E. S. 1948. Physiologic specialization in Guignardia bidwellii cause of black rot of Vitis and Parthenocissus species. Phytopath. 38:716-723.
13. LUTTRELL, E. S. 1951a. The morphology of Dothidea collecta. Am. Jr. Bot., 38:460-471.
14. LUTTRELL, E. S. 1951b. Taxonomy of the Pyrenomycetes. Univ. Mo. Stud., 24, No. 3. 120 pp. University of Missouri, Columbia.
15. LUTTRELL, E. S. 1955. The ascostromatic Ascomycetes. Mycologia, 47:511-532.
16. LUTTRELL, E. S. 1960. The morphology of an undescribed species of Dothiora. Mycologia, 52:64-79.
17. MARTIN, G. W. 1961. Key to the families of fungi. In Dictionary of the Fungi, pp. 497-517. G. C. Ainsworth. Commonwealth Mycological Institute, Kew Surrey.
18. MILLER, J. H. 1949. A revision of the classification of the Ascomycetes with special emphasis on the Pyrenomycetes. Mycologia, 41:99-127.
19. MUNK, A. 1957. Danish pyrenomycetes. Dansk Bot. Ark., 17, 1, 1-491.
20. REDDICK, D. 1911. The black rot disease of grapes. Cornell Bull., 293:289-364.
21. SNYDER, W. C. 1947. Spermogonia versus pycnidia in Mycosphaerella brassicicola. Phytopath., 36:481-484.
22. THEISSEN, F., and H. SYDOW. 1915. Die Dothideales. Ann. Mycol., 13:149-746.
23. THEISSEN, F., and H. SYDOW. 1917. Synoptische Tafeln. Ann. Mycol., 15:389-491.

F. GLOSSARY:

1. Ascostroma (pl. ascostromata; Gr. askos = sac + stroma = cushion, mattress): a stromatic ascocarp bearing asci directly in locules within the stroma.
2. Bitunicate (L. bis = twice + tunica = coat, mantle): an ascus in which the inner wall is elastic and expands greatly beyond the outer wall at the time of spore liberation.
3. Pseudothecium (pl. pseudothecia; Gr. pseudo = false + theke = container): a uniloculate ascostroma.
4. Pycnosclerotium (pl. pycnosclerotia; pycnidium + sclerotium): a more or less hard-walled structure resembling a pycnidium but containing no spores.
5. Spermogonium (pl. spermogonia; Gr. sperma = seed, sperm + gennao = I beget): a structure resembling a pycnidium which contains minute, bacillary microconidia which, in some species, have proved to be functional spermatia.

Class Ascomycetes - Sub-Class Loculoascomycetidae

Order: Pleosporales

A. GENERAL REMARKS:

1. In the Pleosporales, the bitunicate asci develop among pseudoparaphyses and grow upward. The ascocarp is typically a pseudothecium.

2. The order is sub-divided into several families. Only two of these, the Pleosporaceae and the Venturiaceae are included in this manual.

B. PROCEDURE:

Family: Pleosporaceae

Genera: Pleospora, Pyrenophora, Cochliobolus, Ophiobolus, Leptosphaeria

3. Study a species of Pleospora of the P. herbarum type. Note the color of the mycelium and the characteristic septation of conidia and ascospores. These are muriform spores. The imperfect stages of Pleospora herbarum and related species belong to the form-genus Stemphylium.

4. Examine the pseudothecia of any other genera in this family which are made available to you and compare with Pleospora.

Family: Venturiaceae

Genus: Venturia

5. In the Venturiaceae the ascospores are typically unequally two-celled, yellowish-brown, and transparent at maturity. The pseudoparaphyses may disappear by the time the ascospores are mature.

6. Make a complete study of Venturia inaequalis, the cause of apple scab.

   a. If apple leaves with mature, living ascocarps are available, isolate the organism in pure culture in the following manner: Take a sterile Petri dish containing 10 mls. corn meal agar. Place a disc of filter paper (9 cm. diam.) inside the cover of the dish and flatten a wet apple leaf against it with the ascocarps on the side away from the filter paper. Replace the cover on the Petri dish and allow to stand. Examine the surface of the agar periodically for germinating ascospores. These will yield pure cultures of the organism if they are carefully transferred to agar slants or dishes with agar.

   b. Somatic hyphae: Study the mycelium of the fungus from culture. Note the color of the colony. Mount some hyphae and observe the cross-walls and the branching. Note the color of the hyphae.

   c. Conidial stage: Scrape the surface of an infected portion of an apple leaf or apple fruit and mount the scrapings in water or lacto-phenol. Note the shape

and color of the conidia. Study stained cross sections of infected apple fruit tissue which is producing conidia. Note the mycelial mat below the cuticle from which the conidiophores arise. This stage is called Spilocaea pomi. Observe germinating conidia. Note that a septum is generally formed before a conidium puts out a germ-tube.

d. Ascigerous stage: Using a dissecting microscope search an infected over-wintered apple leaf for ascocarps. Note the bristles around the mouth of the ascocarp. Dig out an ascocarp as free from host tissue as possible, place it in water and gently lower a cover-glass over it. Examine under the low and high power objectives applying gentle pressure on the cover-glass, if necessary, by means of a mounted needle. Study the two-celled ascospores within the asci which you have forced out of the ascocarp. Study stained sections of ascocarps. Note the pseudoparaphyses among the asci.

C. QUESTIONS:

1. Characterize the Pleosporales. How do they differ from the Dothideales?
2. How do the pseudoparaphyses of the Pleosporales originate?
3. How do the Pleosporaceae differ from the Venturiaceae?
4. Is Venturia inaequalis a parasite or a saprobe? Explain.
5. What are the ring-like scars near the tips of the conidiophores of Venturia inaequalis?

D. TEXT REFERENCES:

Alexopoulos -- pp. 377-381.
Bessey -- pp. 295-296; 298-299.

E. SELECTED REFERENCES:

1. BOONE, D. M. and G. W. KEITT. 1957. Venturia inaequalis (Cke.) Wint. XII. Genes controlling pathogenicity of wild-type lines. Phytopath., 47:403-409.
2. HOLM, L. 1957. Études taxonomiques sur les Pleosporacées. Symb. Bot. Upsalienses, 14, 3. 188 pp.
3. JOHNSON, T. W., JR. 1956. Marine fungi. I. Leptosphaeria and Pleospora. Mycologia, 48:495-505.
4. KERR, JANET E. 1961. The life history and taxonomic position of Venturia rumicis (Desm.) Wint. Trans. Brit. Mycol. Soc., 44:465-486.
5. KNOX-DAVIES, P. S. and J. G. DICKSON. 1960. Cytology of Helminthosporium turcicum and its ascigerous stage Trichometasphaeria turcica. Am. Jr. Bot., 47:328-338.
6. LUTTRELL, E. S. 1955. The ascostromatic Ascomycetes. Mycologia, 47:511-532.
7. MÜLLER, E. 1950. Die schweizerischen Arten der Gattung Leptosphaeria and ihrer Verwandten. Sydowia, Ann. Mycol., II. 4:185-319.
8. MÜLLER, E. 1951. Über die Entwicklung von Pleospora gauemannii nov. spec. Ber. schweiz. bot. Gesell., 61:165-174.
9. MÜLLER, E. and J. A. VON ARX. 1950. Einige Aspekte zur Systematik pseudosphaerialer Ascomyceten. Ber. schweiz. bot. Gesell., 60:329-397.
10. PLAKIDAS, A. G. 1942. Venturia acerina, the perfect stage of Cladosporium humile. Mycologia, 34:27-37.
11. von ARX, J. A. 1952. Studies on Venturia and related genera. Tijd. Plantenziekt. 58:260-266.
12. WEBSTER, J. 1955a. Graminicolous pyrenomycetes. V. Conidial states of Leptosphaeria michotii, L. microscopica, Pleospora vagans, and the perfect state of Dinemasporium graminum. Trans. Brit. Mycol. Soc., 38:347-365.
13. WEBSTER, J. 1955b. Hendersonia typhae, the conidial state of Leptosphaeria typharum. Trans. Brit. Mycol. Soc., 38:405-408.
14. WEHMEYER, L. E. 1961. A world monograph of the genus Pleospora and its segregates. ix + 451 pp. Illus. University of Michigan Press, Ann Arbor.

F. GLOSSARY:

1. Muriform (L. murus = wall + forma = form): divided into many cells by means of longitudinal as well as of transverse septa.

Class Ascomycetes – Sub-Class Loculoascomycetidae

Order: Hysteriales

A. GENERAL REMARKS:

1. The ascocarp of the Hysteriales is a black, boat-shaped, ascostroma with an elongated slit-like opening, and is given the special designation of hysterothecium.

2. According to the most recent view, the order Hysteriales contains only one family: the Hysteriaceae. However, lichen fungi with hysterothecia are also placed in this order in one or more separate families.

B. PROCEDURE:

Family: Hysteriaceae

Genera: Glonium, Hysterium, Hysterographium

3. Using a dissecting microscope examine dead twigs, branches, or pieces of bark from decaying logs on which hysterothecia are found. Note the tendency toward crowding, the shape of the individual ascocarps and the slit-like opening at the top. The ascocarps of the Hysteriaceae are superficial.

4. Cut a thin cross-section through an ascocarp of each of the three genera listed above, and examine microscopically in a lacto-phenol mount. Note the two-celled ascospores of Glonium, the many-celled ascospores of Hysterium, and the muriform ascospores of Hysterographium.

C. QUESTIONS:

1. Characterize the Hysteriales.
2. To what other order are the Hysteriales thought to be related?
3. What family besides the Hysteriaceae was formerly classified (and still is by some authors) in the Hysteriales? To what order has it been transferred? (Bessey, 1950; Dennis, 1960).
4. What types of conidial stages, if any, have been found to belong to the Hysteriales?

D. TEXT REFERENCES:

Alexopoulos -- pp. 381-382.
Bessey -- pp. 239-241.

E. SELECTED REFERENCES:

1. BISBY, G. R. 1913. The literature on the classification of the Hysteriales. Trans. Brit. Mycol. Soc., 8:176-189.
2. DENNIS, R. W. G. 1960. British cup fungi and their allies. xxiv + 280 pp. 20 figs. 40 col. pls. The Ray Society, London.
3. LOHMAN, M. L. 1933. Hysteriaceae: Life histories of certain species. Papers Mich. Acad. Sci. Arts and Lett., 17:229-288.
4. LOHMAN, M. L. 1937. Studies in the genus Glonium as represented in the southeast. Bull. Torrey Bot. Club, 64:57-72.
5. LUTTRELL, E. S. 1953. Development of the ascocarp in Glonium stellatum. Am. Jr. Bot., 40:626-633.

F. GLOSSARY:

1. Hysterothecium (pl. hysterothecia; Gr. hysteros = womb + theke = box): a boat-shaped ascocarp with an elongated, slit-like opening at the top.

FORM-CLASS DEUTEROMYCETES

VARIOUS TYPES OF SPORES

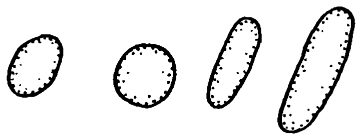


FIG. 54 HYALOSPORAE

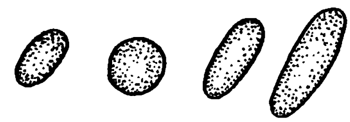


FIG. 55 PHAEOSPORAE

AMEROSPORAE

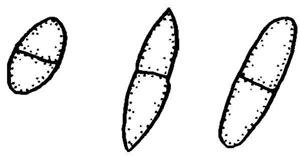


FIG. 56 HYALODIDYMAE

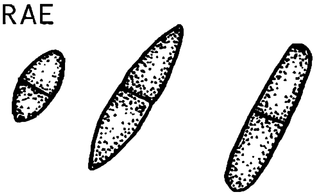


FIG. 57 PHAEODIDYMAE

DIDYMOSPORAE

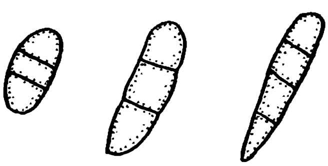


FIG. 58 HYALOPHRAGMIAE

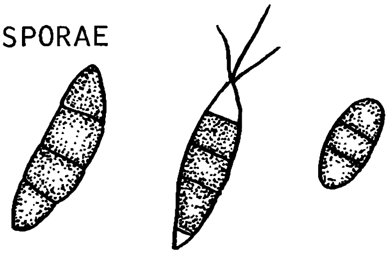


FIG. 59 PHAEOPHRAGMIAE

PHRAGMOSPORAE

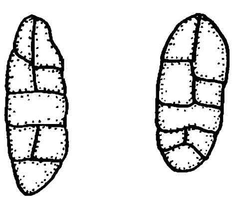


FIG. 60 HYALODICTYAE

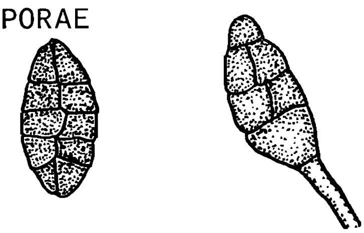


FIG. 61 PHAEODICTYAE

DICTYOSPORAE

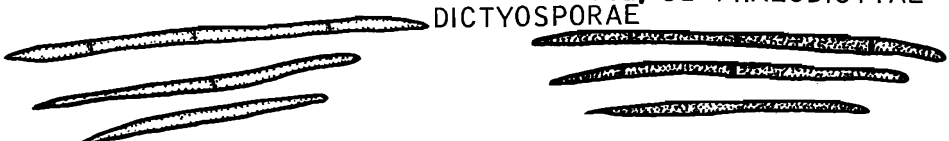


FIG. 62 SCOLECOSPORAE

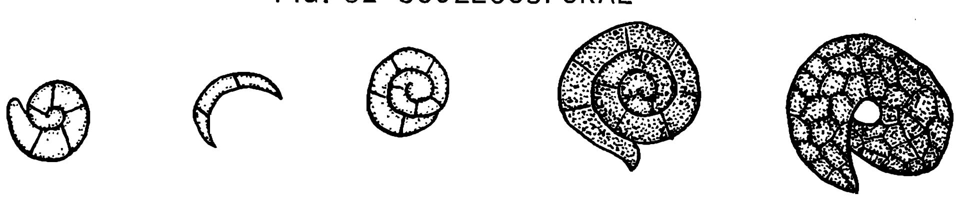


FIG. 63 HELICOSPORAE

Plate 13

Form-Class Deuteromycetes

A. GENERAL REMARKS:

1. The Deuteromycetes (Fungi Imperfecti) are a group of fungi whose sexual (perfect) stages, if indeed they exist, have not been found. Reproduction takes place, characteristically, by means of conidia of various types or chlamydospores. A parasexual cycle has been demonstrated in several form-species and it is probable that this mechanism is widely prevalent in this group of fungi and takes the place of true sexuality.

2. The classification of the Deuteromycetes presently in use is an entirely artificial system based on the color, shape, and size of the conidia and conidiophores. Other characters used in taxonomy are those of the more elaborate fruiting bodies, such as pycnidia, acervuli, synnemata, and sporodochia which serve to distinguish among form-orders. Because this classification does not necessarily indicate relationships, the Deuteromycetes are designated as a form-class.

3. It appears now that a more natural system of classification will arise from the studies of Hughes (1953, 1958) and others, but much more detailed work is required before such a system replaces the present one.

4. Many of the Fungi Imperfecti cause serious diseases of plants, animals, and man. The majority, however, are saprobic.

5. The Deuteromycetes are subdivided into four form-orders: the *Sphaeropsidales*, the *Melanconiales*, the *Moniliales* and the *Mycelia Sterilia*.

6. The Sphaeropsidales produce their conidia on simple or branched conidiophores borne in pycnidia. This order includes four form-families only one of which, the *Sphaeropsidaceae*, will be studied.

B. PROCEDURE:

Form-Order: Sphaeropsidales

Form-Family: Sphaeropsidaceae

Section: Amerosporae - conidia one-celled

Sub-Section: Hyalosporae - conidia hyaline

Form-Genera: Phyllosticta, Phoma, Phomopsis, Dendrophoma

7. You have already studied *Phyllosticta labruscae* (the imperfect stage of *Guignardia bidwellii*) the cause of black rot of grapes.

8. *Dendrophoma obscurans* causes leaf blight of strawberries. Pick out a pycnidium as free from host tissue as possible and mount in lacto-phenol. Note the long, branched conidiophores which become evident when the pycnidium is crushed. Observe the beak of the pycnidium, the ostiole, and the hyaline, elongated conidia.

9. You have already studied the characteristics of the form-genus *Phomopsis* under the ascomycete genus *Diaporthe*, of which *Phomopsis* is the imperfect form.

Sub-Section: Phaeosporae - conidia colored

Form-Genera: Sphaeropsis, Coniothyrium

10. Study a species of Coniothyrium, such as Coniothyrium diplodiella and compare with Sphaeropsis. The only difference between these two form-genera is the size of the conidia.

Section: Didymosporae - conidia two-celled

Sub-Section: Hyalodidymae - conidia hyaline

Form-Genus: Ascochyta

11. Study any species of Ascochyta, such as Ascochyta pisi. Mount a pycnidium in water or lacto-phenol and observe the two-celled, hyaline conidia.

Sub-Section: Phaeodidymae - conidia colored

Form-Genus: Diplodia

12. Study a species of Diplodia, such as Diplodia zeae on corn. Note the two-celled brown conidia.

Section: Scolecosporae - conidia hyaline or greenish, needle-shaped to filiform

Form-Genus: Septoria

13. Septoria apii is the cause of late blight of celery. Study the pycnidia and conidia from an infected leaf. Note the thread-shaped conidia with septa. Study other species of Septoria and compare.

14. Study representatives of as many other genera of the Sphaeropsidales as time and material permit and learn to recognize the common genera.

C. QUESTIONS:

1. What are the Deuteromycetes? What is another name commonly applied to these fungi?
2. Name the form-orders of the Deuteromycetes and briefly distinguish among them.
3. What are some of the characters used in the classification of the Deuteromycetes? Why is this "class" considered an artificial group?
4. Explain Saccardo's system of classification of the Deuteromycetes.
5. Explain the newer system proposed by Hughes.
6. Describe the parasexual cycle in the fungi. Of what importance is parasexuality to the fungi which exhibit this mechanism?
7. What is a pycnidium? How does a pycnidium develop?
8. Construct a key to the form-genera of the Sphaeropsidales you have studied.
9. Name some important plant diseases caused by members of the Sphaeropsidales and give the causal agent in each case.

D. TEXT REFERENCES:

Alexopoulos -- pp. 387-404.
Bessey -- pp. 572-581.

E. SELECTED REFERENCES:

1. BARNETT, H. L. 1960. Illustrated genera of imperfect fungi. iii + 225 pp. 462 figs. Burgess Publishing Co. Minneapolis.
2. BENDER, H. B. 1931. The genera of Fungi Imperfecti: North American species and hosts, with particular reference to Connecticut. Thesis Yale University. 2000 pp. (Unpublished).
3. BENDER, H. B. 1934. The Fungi Imperfecti: Order Sphaeropsidales. 52 pp. North Woodbury, Conn. Published by the author.
4. BENEKE, E. S. 1957. Medical mycology laboratory manual. v + 186 pp. 29 figs. 14 pls. (Many in color) Burgess Publishing Co., Minneapolis.
5. BENHAM, RHODA W. 1931. Phoma conidiogena, an excitant of asthma: some observations on the development and cultural characteristics. Bull. Torrey Bot. Club, 58:203-214.
6. BREWER, D. 1960. Studies in Ascochyta pisi Lib. Can. Jr. Bot., 38:705-717.
7. CLEMENTS, F. E. and C. L. SHEAR. 1931. The genera of fungi. iv + 496 pp., 58 pls. H. W. Wilson Co., New York.
8. CROSSAN, D. F. 1958. The relationships of seven species of Ascochyta occuring in North Carolina. Phytopaht., 48:248-255.
9. ELLINGBOE, A. H. 1959. Studies on the growth of Phoma herbarum var. medicaginis in culture. Phytopath., 49: 773-776.
10. GILMAN, J. C. 1957. Soil fungi. x + 450 pp. 153 figs. Frontisp. Iowa State University Press, Ames.
11. GILMAN, J. C. and B. N. WADLEY. 1952. The ascigerous stage of Septoria quercini Thuem. Mycologia, 44: 216-220.
12. GROVE, W. B. 1935. British stem and leaf fungi (Coelomycetes). Vol. I. Sphaeropsidales. xx + 488 pp. 31 figs. Cambridge University Press. Cambridge.
13. GUBA, E. F. 1925. Phyllosticta leaf spot, fruit blotch, and canker of the apple; its etiology and control. Ill. Agr. Exp. Sta. Bull. 256:481-551.
14. HANSEN, H. N. 1938. The dual phenomenon in imperfect fungi. Mycologia, 30:442-455.
15. HOOKER, A. L. 1957. Cultural variability in Septoria avenae through successive single-macrospore transfers. Phytopath., 47:460-468.
16. HUGHES, S. J. 1953. Conidiophores, conidia, and classification. Can. Jr. Bot., 31:577-659.
17. HUGHES, S. J. 1958. Revisiones hyphomycetum aliquot cum appendice de nominibus rejiciendis. Can. Jr. Bot., 36:727-836.
18. JOHNSON, T. 1952. Cultural variability in Septoria avenae Frank. Can. Jr. Bot., 30:318-330.
19. KEMPTON, F. E. 1919. Origin and development of the pycnidium. Bot. Gaz. 68:233-261.
20. PONTECORVO, G. 1956. The parasexual cycle in fungi. Ann. Rev. Microbiol., 10:393-400.
21. PONTECORVO, G. 1958. Trends in genetic analysis. 145 pp. Illustr. Columbia University Press, New York.
22. SACCARDO, P. A. 1899. Sylloge fungorum omnium hucusque cognitorum. Vol. 14. 1316 pp. Published by the author. Pavia, Italy.
23. STEVENS, F. L. 1913. The fungi which cause plant disease. ix + 754 pp. 449 figs. The Macmillan Co., New York.

F. GLOSSARY:

1. Parasexual Cycle (Gr. para = beside + sexual): a nuclear cycle in which plasmogamy, karyogamy, and haploidization occur, but not in a specified organ nor at a definite time in the life history of the organism.
2. Pycnidiospore (pycnidium + Gr. sporos = seed, spore): a conidium produced in a pycnidium.
3. Synnema (pl. synnemata; Gr. syn = together + nema = skein): a group of conidiophores cemented together and forming an elongated, spore-bearing structure.

Form-Class Deuteromycetes

Form-Order: Melanconiales

Form-Family: Melanconiaceae

A. GENERAL REMARKS:

1. In the Melanconiales the short conidiophores are produced in an acervulus. All members of this order are assembled in the single form-family Melanconiaceae. Fungi in this form-family cause plant diseases which are designated as "anthracnoses".

B. PROCEDURE:

Section: Amerosporae - conidia one-celled

Sub-Section: Hyalosporae - conidia hyaline

Form-Genera: Gloeosporium, Colletotrichum

2. These two genera of the Melanconiales are very common on a number of plant hosts. The only difference between the two genera is that Colletotrichum usually produces dark brown to black setae in the acervulus, whereas Gloeosporium usually does not.

3. Study any common species of Gloeosporium such as Gloeosporium rufomaculans (the imperfect stage of Glomerella singulata) from culture or from the host. Note the pink masses of conidia on the surface of the acervulus. Mount some conidia in water and study under the high power objective. Note the characteristic oil drops which are generally abundant in the conidia. Note the shape and size of the conidia. Observe that no setae are present in the acervulus.

4. Observe a culture of Colletotrichum lindemuthianum under the high power of the binocular dissecting microscope. Note the bristle-like, brown or black, setae in each acervulus. Observe the color of the conidial masses. Mount a portion of the acervulus from the culture or a section through an acervulus on a bean pod or stalk. Study the setae and the conidia. Compare and contrast with Gloeosporium.

Sub-Section: Phaeosporae - conidia colored

Form-Genus: Melanconium

5. Study the acervuli of Melanconium fuligineum, the cause of grape bitter rot, from culture or from an infected grape berry. Observe that the acervuli are not very different from pycnidia. Mount some conidia in water and note their shape and smoky color.

Section: Didymosporae - conidia two-celled

Sub-Section: Hyalodidymae - conidia hyaline

Form-Genus: Marssonina

6. Several species in this form-genus cause diseases of economic plants. You have already studied Marssonina juglandis, the imperfect stage of Gnomonia leptostyla; Marssonina rosae is the imperfect stage of Diplocarpon rosae (Helotiales, Dermataceae); Marssonina fragariae is the imperfect stage of Diplocarpon earliana.

Section: Phragmosporae - conidia two- to many-celled

Sub-Section: Hyalophragmiae - conidia hyaline

7. Entomosporium maculatum, which you have already studied as the imperfect stage of Diplocarpon soraueri, on pear and quince, belongs here. Some taxonomists, however, regard the asexual fruiting body of this fungus to be a flattened pycnidium and place the form-genus Entomosporium in the Shpaeropsidales (form-family Leptostromataceae).

Sub-Section: Phaeophragmiae - conidia dark

Form-Genus: Pestalotia

8. This is a large form-genus of some 100 species, some of them of economic importance. Study a culture of Pestalotia guepinii or other form-species available and note the black, spore masses over the acervuli. Mount some conidia in water and observe under high power or oil immersion objective. Note the characteristic hair-like appendages (setae) of the conidia, and the distribution of the smoky pigment in the conidial cells.

Section: Scolecosporae - conidia cylindric, filiform or somewhat curved; hyaline

Form-Genus: Cylindrosporium

9. You have studied Cylindrosporium padi, the imperfect stage of Higginsia hiemalis (Helotiales, Dermataceae). Other form-species of Cylindrosporium are C. pomi on apple, and C. chrysanthemi on chrysanthemum.

C. QUESTIONS:

1. Characterize the Melanconiales.
2. How does an acervulus differ from a pycnidium?
3. Construct a key to the genera of the Melanconiales you have studied.
4. Name some important plant diseases caused by members of this group.

D. TEXT REFERENCES:

Alexopoulos -- pp. 404-407.
Bessey -- pp. 581-583.

E. SELECTED REFERENCES:

1. EDGERTON, C. W. 1912. The Melanconiales. Trans. Amer. Microsc. Soc. 31:243-265.
2. GROVE, W. B. 1937. British stem and leaf fungi (Coelomycetes). Vol. II. Sphaeropsidales and Melanconiales. xii + 407 pp. 133 figs. Cambridge University Press, Cambridge.
3. GUBA, E. F. 1961. Monograph of Monochaetia and Psstalotia. vii + 342 pp. 125 figs. Harvard University Press, Cambridge.
4. LENTZ, P. L. 1950. The genus Marssonina on Quercus and Castanea. Mycologia, 42:259-264.
5. MATHUR, R. S., H. L. BARNETT, and V. G. LILLY. 1950. Sporulation of Colletotrichum lindemuthianum in culture. Phytopath., 40:104-114.
6. MISRA, A. P. and M. MAHMOOD. 1960. Effect of carbon and nitrogen nutrition on growth and sporulation of Colletotrichum capsici (Syd.) Butler and Bisby. Ind. Bot. Soc. Jr., 2:314-321.
7. SHEAR, C. L. and ANNA K. WOOD. 1907. Ascogenous forms of Gloeosporium and Colletotrichum. Bot. Gaz., 43:259-266.
8. TIMNICK, MARGARET B., H. L. BARNETT, and V. G. LILLY. 1952. The effect of method of inoculation of media on sporulation of Melanconium fuligineum. Mycologia, 44:141-149.
9. TIMNICK, MARGARET, B., V. G. LILLY, and H. L. BARNETT. 1951. The effect of nutrition on the sporulation of Melanconium fuligineum in culture. Mycologia, 43:625-634.
10. von ARX, A. J. 1957. Revision die zu Gloeosporium Gestellten Pilze. Verhandl. Koninkl. Nederl. Akad. Wetensch. Afd, Natuurk., 51(3):1-153.

Form-Class Deuteromycetes

Form-Order: Moniliales

A. GENERAL REMARKS:

1. The Moniliales produce their conidia directly upon the hyphae or upon specialized conidiophores. The conidiophores may be simple or branched and solitary, or clustered together forming sporodochia or synnemata. No pycnidia or acervuli develop. Many of the important diseases of plants and most of the fungous diseases of animals and man are caused by organisms which belong to the Moniliales.

2. The form-order is subdivided into five form-families; four of these (Moniliaceae, Dematiaceae, Tuberculariaceae, and Stilbellaceae) include mycelial fungi; the other (Cryptococcaceae) includes the false yeasts and yeast-like organisms. We shall study only a few representatives of the first four form-families.

B. PROCEDURE:

Form-Family: Moniliaceae - hyphae in loose cottony masses; hyphae and conidia hyaline or bright colored

Section: Amerosporae - conidia one-celled

Form-Genera: Botrytis, Geotrichum, Verticillium, Aspergillus, Penicillium

3. The form-genus Botrytis includes several economically important plant parasites. B. paeoniae causes gray mold blight of peony; B. cinerea is frequently parasitic on a number of plants; B. tulipae causes a serious blight of tulips.

4. Observe the conidiophores and conidia of any species of Botrytis under the high power of the binocular dissecting microscope for a picture of the general aspect of the fungus. Carefully mount a conidiophore in lacto-phenol containing cotton blue, and study under the microscope. Observe the branching of the conidiophore and the sterigmata which bear the conidia. Note the size and shape of the conidia.

5. Various species of Geotrichum are associated with geotrichosis, a fungous disease of man. Some species as Geotrichum candidum (Oidium lactis, Oöspora lactis) are important saprobes which destroy foodstuffs. Study a culture of this organism. Note the cuboid arthrospores.

6. Verticillium is a form-genus including important plant pathogens which cause a wilting of their hosts. Study Verticillium albo atrum. Note the color of the colony. Observe the verticillately branched conidiophores from which the form-genus gets its name.

7. Aspergillus and Penicillium belong to this form-family. You have already studied these, in connection with their perfect stages, under the Plectomycetes.

Section: Didymosporae - conidia two-celled

Form-Genus: Trichothecium

8. Trichothecium roseum is a common saprobe and weak parasite. Sometimes it causes considerable trouble as a secondary invader of apple tissue following apple scab, and causing "pink rot". Study the characteristically septate conidia and, if possible, the method of their attachment. You can best see the whole conidial apparatus if you observe a colony of the fungus under the high power of a good binocular dissecting microscope.

Section: Phragmosporae - conidia many-celled; septa transverse

Form-Genus: Epidermophyton

9. Epidermophyton is one of the three important form-genera causing fungous infections of the superficial skin. They are known as dermatophytes. Examine a culture of E. floccosum, a frequent cause of athlete's foot. Observe the large, clavate, multiseptate, smooth, thin-walled conidia (macro-conidia). The hyphae also produce chlamydospores which you will recognize as swollen cells in the hyphae.

10. If you suspect an infection of athlete's foot, mount a small portion of the inflamed skin in 10% KOH. Look for segmented mycelial elements in the epithelial cells.

Form-Family: Dematiaceae - hyphae in loose cottony masses; hyphae and conidia both dark, or only one may be dark

Section: Amerosporae - conidia one-celled

Form-Genus: Hormodendrum

11. This is a common form-genus containing some saprobes and some important human parasites. Among the latter is H. pedrosoi. The conidia of some species may be important allergens. Study any species available and learn the characters of the genus.

Section: Didymosporae - conidia two-celled

Form-Genus: Cladosporium

12. Many mycologists regard Cladosporium and Hormodendrum as synonymous. The only difference between the two is that Cladosporium has two-celled conidia whereas Hormodendrum has one-celled conidia. However, both one- and two-celled conidia are often formed by the same mycelium.

Section: Phragmosporae - conidia many-celled; septa transverse

Form-Genera: Helminthosporium, Heterosporium

13. Several species of Helminthosporium cause important diseases of cereal crops and wild and cultivated grasses, called stripe diseases. The conidiophores are simple and septate. The conidia are attached at the tip and the sides of the conidiophores on small protrusions. Mount some conidiophores with conidia and study the method of conidial attachment and the characteristic shape, color, and septation of the conidia. Compare with Epidermophyton.

14. Study Heterosporium iridis on infected Iris leaves. Note that the conidia of this form-genus are spiny or warty in contrast to those of Helminthosporium which are smooth.

Section: Dictyosporae - conidia many-celled; muriform

Form-Genera: Alternaria, Stemphylium

15. Alternaria is a common inhabitant of house dust. Some species are parasitic, causing important plant diseases; others are saprobic. Study a culture of Alternaria under the binocular dissecting microscope. Observe the long chains of rather large conidia. Mount a small portion of the colony in water and study the mycelium and the conidia. Observe the great variation in number and arrangement of septa.

16. Stemphylium produces dark muriform conidia which are borne singly rather than in chains. You have studied this form-genus as the imperfect stage of Pleospora herbarum (Pleosporales, Pleosporaceae).

Section: Scolecosporae - conidia needel-shaped to filiform

Form-Genus: Cercospora

17. Study a species of Cercospora, such as Cercospora apii on celery. Observe the relatively short conidiophores and the long, many septate conidia.

Section: Helicosporae

Form-Genus: Helicosporium

18. Study any available species in this or other genus of the Helicosporae. Observe the interesting, coiled conidia borne on conidiophores.

Form-Family: Tuberculariaceae - hyphae compacted into a more or less globose, or cushion-shaped stoma, a sporodochium

Section: Amerosporae - conidia one-celled

Sub-Section: Hyalosporae - conidia hyaline

Form-Genus: Tubercularia

19. Tubercularia vulgaris, the imperfect stage of Nectria cinnabarina which you studied in the Hypocreales, belongs here.

Section: Phragmosporae - conidia many-celled

Sub-Section: Hyalophragmiae - conidia hyaline

Form-Genus: Fusarium

20. Fusarium contains a number of form-species which are important plant pathogens, and many which are saprobes. The parasitic species generally cause a wilt or a rot of the host. Study a culture of Fusarium which produces both macroconidia and microconidia. The macroconidia are fusoid, curved, and generally

many septate. The microconidia may be similar to the macroconidia but smaller, or they may be of a distinct type. Mount a portion of the mycelium in water and note the large numbers of chlamydospores. Recall that you have studied Fusarium as the imperfect stage of Gibberella (Hypocreales, Nectriaceae).

Sub-Section: Phaeosporae - conidia dark

Form-Genus: Epicoccum

21. Study any form-species of Epicoccum available. Note the sporodochia and the globoid, ridged or septate conidia with rough walls, attached to short, club-shaped conidiophores.

Form-Family: Stilbellaceae

Section: Hyalostilbeae-Amerosporae - hyphae and conidia hyaline; conidia one-celled

Form-Genus: Isaria

22. Isaria is a large form-genus consisting mostly of entomogenous fungi. If available, study the synnemata of any representative of this interesting form-genus. Note the branching of the conidiophores, and the attachment of the conidia.

Section: Phaeostilbeae-Amerosporae - hyphae or conidia dark; conidia one-celled

Form-Genus: Graphium

23. Study the synnemata (coremia) of Graphium ulmi or other species of Graphium. Observe the structure of the synnema and the method of conidial attachment.

Form-Order: Mycelia Sterilia

A. GENERAL REMARKS:

24. The Mycelia Sterilia include fungi known only from their mycelial stages. No conidia or other spore forms are produced. In the few instances in which perfect stages have been found, these have proved to be Basidiomycetes.

Form-Genera: Rhizoctonia, Sclerotium

25. Study the mycelium of a culture of Rhizoctonia. Note the characteristic branching of the hyphae.

26. Study a culture of Sclerotium rolfsii. Observe the thick strands of white mycelium and the small globose, sclerotia typical of this fungus. Section a sclerotium and study the internal structure.

C. QUESTIONS:

1. Construct a key to the form-genera of the Moniliales which you have studied.
2. List some important plant diseases caused by the Moniliales and name the causal organism for each.

3. List some important animal and human diseases caused by members of the Moniliales and name the causal organism for each.

D. TEXT REFERENCES:

Alexopoulos -- pp. 407-418.
Bessey -- pp. 583-602.

E. SELECTED REFERENCES:

1. BENDER, H. B. 1931. The genera of Fungi Imperfecti. North American species and hosts, with particular reference to Connecticut. 2000 pp. Thesis. Yale University, New Haven. (Unpublished)
2. BENHAM, RHODA W. 1956. The genus Cryptococcus. Bact. Rev., 20:189-201.
3. BUXTON, E. W. 1956. Heterokaryosis and parasexual recombination in pathogenic strains of Fusarium oxysporum. Jr. Gen. Microbiol., 15:133-139.
4. BUXTON, E. W. 1959. Mechanisms of variation in Fusarium oxysporum in relation to host-parasite interactions. In plant pathology; problems and progress, 1908-1958, pp. 183-191. American Phytopathological Society.
5. CONANT, N. F. ET AL. 1954. Manual of clinical mycology. xii + 456 pp. 202 figs. Frontisp. W. B. Saunders Co., Philadelphia.
6. COOKE, W. B. 1959. An ecological life history of Aureobasidium pullulans (de Bary) Arnaud. Mycopath. Mycol. Appl., 12:1-45.
7. DUDDINGTON, C. L. 1957. The friendly fungi. 188 pp. 7 figs. 24 pls. Faber and Faber, London.
8. ELLIOTT, J. A. 1917. Taxonomic characters of the genera Alternaria and Macrosporium. Am. Jour. Bot., 4:439-476.
9. EPPS, W. M., J. C. PATTERSON, and I. E. FREEMAN. 1951. Physiology and parasitism of Sclerotium rolfsii. Phytopath. 41:245-256.
10. GEORG, LUCILLE K. 1960. Epidemiology of the dermatophytoses: sources of infection, modes of transmission, and epidemicity. Ann. N. Y. Acad. Sci., 89:69-77.
11. GRIGORAKI, L. 1956. Les champignons parasites des teignes. Mycopath. et Mycol. Appl., 7:291-320.
12. GROVES, J. W. and A. J. SKOLKO. 1944a. Notes on seed-borne fungi. I. Stemphylium. Can. Jr. Res., C22: 190-199.
13. GROVES, J. W. and A. J. SKOLKO. 1944b. Notes on seed-borne fungi. II. Alternaria. Can. Jr. Res., C22: 217-234.
14. HUGHES, S. J. 1953. Conidiophores, conidia, and classification. Can. Jr. Bot., 31:577-659.
15. HUGHES, S. J. 1958. Revisiones hyphomycetum aliquot cum appendice de nominibus rejiciendis. Can. Jr. Bot., 36:727-836.
16. INGOLD, C. T. 1956. The conidial apparatus of Trichothecium roseum. Trans. Brit. Mycol. Soc., 39:460-464.
17. JACQUES, J. E. 1941. Studies in the genus Heterosporium. Institute of Botany, University of Montreal. 46 pp.
18. JOHNSON, E. M. and W. D. VALLEAU. 1949. Synonymy in some common species of Cercospora. Phytopath., 39:763-770.
19. LEWIS, G. M. and M. E. HOPPER. 1948. An introduction to medical mycology. The Year Book Publishers Inc.
20. LINDER, D. H. 1929. A monograph of the helicosporous Fungi Imperfecti. Ann. Mo. Bot. Gard., 16:227-388.
21. LUTTRELL, E. S. 1951. A key to species of Helminthosporium reported on grasses in the United States. U. S. Dept. Agr. Pl. Dis. Rept. Suppl. 201:59-67.
22. LUTTRELL, E. S. 1955. A taxonomic revision of Helminthosporium sativum and related species. Am. Jr. Bot., 42:57-68.
23. LUTTRELL, E. S. 1958. The perfect stage of Helminthosporium turcicum. Phytopath., 48:281-287.
24. MOORE, R. T. 1955. Index to the Helicosporae. Mycologia, 47:90-103.
25. MOORE, R. T. 1958. Deuteromycetes. I. The Sporidesmium complex. Mycologia, 50:681-692.
26. PONTECORVO, G., ET AL. 1953. The genetics of Aspergillus nidulans. Adv. Genetics., 5:141-238.
27. RANZONI, F. V. 1953. The aquatic hyphomycetes of California. Farlowia, 4:353-398.
28. REYNOLDS, E. S. 1950. Pullularia as a cause of deterioration of paint and plastic surfaces in South Florida. Mycologia, 42:432-448.
29. SKINNER, C. E., C. W. EMMONS, and H. M. TSUCHIYA. 1947. Molds, yeasts and actinomycetes. Ed. 2. xiv + 409 pp. 136 figs. John Wiley and Sons, Inc., New York.
30. SNYDER, W. C. and H. N. HANSEN. 1945. The species concept in Fusarium with reference to Discolor and other sections. Am. Jour. Bot., 32:657-666.
31. SNYDER, W. C. and H. N. HANSEN. 1954. Variation and speciation in the genus Fusarium. Ann. N. Y. Acad. Sci., 60:16-23.
32. TUBAKI, K. 1958. Studies in the Japanese hyphomycetes. V. Leaf and stem group with a discussion of the classification of hyphomycetes and their perfect stages. Jr. Hattori Bot. Lab., 20:142-244.
33. WILTSHIRE, S. P. 1933. The foundation species of Alternaria and Macrosporium. Trans. Brit. Mycol. Soc., 18:135-160.

F. GLOSSARY:

1. Arthrospore (Gr. arthron = joint + sporos = spore): a spore resulting from the fragmentation of a hypha at the septa; also called oidium.
2. Coremium (pl. coremia): synonymous with synnema (page 156).

CLASS BASIDIOMYCETES

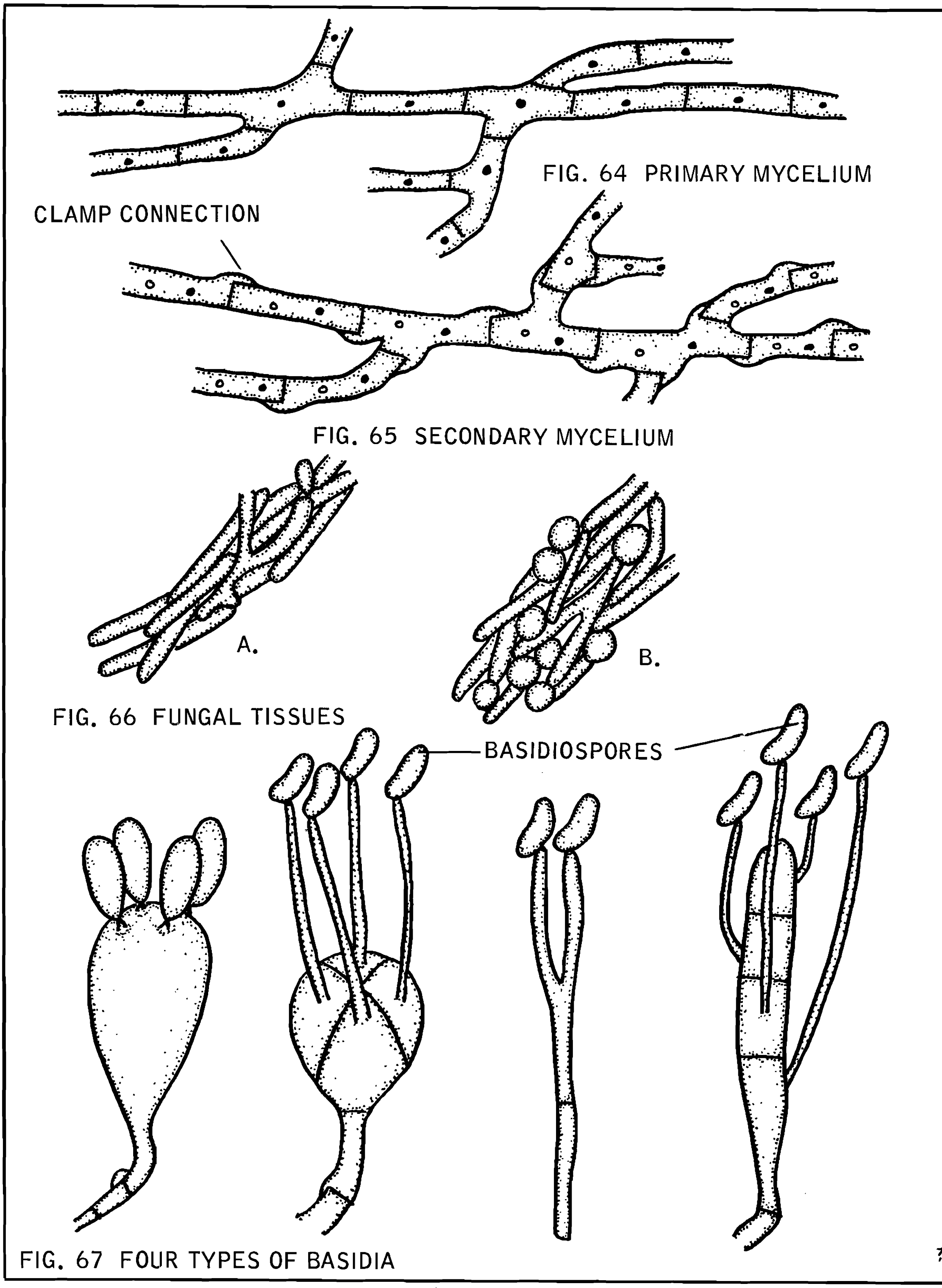


FIG. 64 PRIMARY MYCELIUM

FIG. 65 SECONDARY MYCELIUM

FIG. 66 FUNGAL TISSUES

FIG. 67 FOUR TYPES OF BASIDIA

Plate 14

Class Basidiomycetes–Sub-Class Heterobasidiomycetidae

A. GENERAL REMARKS:

1. The Basidiomycetes are thought to have evolved from an ascomycetous ancestor and are the most advanced of all the fungi. Their characteristic structure is the basidium in which karyogamy and meiosis occur. The basidium bears the basidiospores.

2. Two sub-classes are recognized by most mycologists, but their naming has not been standardized. We shall use the terms Heterobasidiomycetidae and Homobasidiomycetidae to designate them.

3. The basidia of the Heterobasidiomycetidae are either deeply divided, longitudinally or transversely septate, or may consist of a thick-walled teleutospore which produces a promycelium upon which the basidiospores are borne.

4. The Heterobasidiomycetidae include the jelly fungi (Tremellales), the rusts (Uredinales), and the smuts (Ustilaginales).

B. PROCEDURE:

Order: Tremellales

5. The Tremellales or Jelly Fungi, usually have well-developed basidiocarps the consistency of which varies from gelatinous in most species, to waxy, leathery, or even papery. The basidia are variously divided or septate, the nature of the basidium being the chief character on which the families are distinguished.

6. Nine families are recognized by Martin (1961). We shall study five of these.

Family: Dacrymycetaceae

Genera: Dacrymyces, Calocera

7. The Dacrymycetaceae bear their basidiospores on a deeply divided basidium (tuning-fork basidium) which bears only 2 basidiospores.

8. Examine basidiocarps of Dacrymyces deliquescens which have been kept in a moist chamber for a few hours. Note the color and consistency of the basidiocarps.

9. Mount a portion of a young orange colored basidiocarp. Examine the hyphae carefully, find the oidia and observe the method of their formation.

10. Mount a portion of a mature basidiocarp. Search for clamp connections in the mycelium. Find the characteristic "tuning-fork" basidia. Note the pointed sterigmata and the curved basidiospores.

11. If basidiospores are present in sufficient quantities make a hanging drop preparation of basidiospores in distilled water and study the method of germination on the following laboratory period.

12. Examine mature basidiocarps of Calocera cornea. Basidia and basidiospores are very similar to those of Dacrymyces. If time permits mount a portion of the fructification and study these structures.

C. QUESTIONS:

1. Characterize the Basidiomycetes.
2. Describe the development of clamp connections.
3. Describe the nuclear history of the basidium.
4. Describe the formation and method of dispersion of basidiospores as exemplified by Calocera cornea (Consult Buller, 1922).
5. To what ascomycetous structures are the following homologous: basidium, basidiospore, binucleate mycelium? Give reasons for your answer.
6. What is a hypobasidium? An epibasidium? A probasidium?
7. Are these terms applicable to the Dacrymycetaceae? (Consult Rogers (1934), Martin (1938), Bessey (1950) for various views.)
8. Characterize the Heterobasidiomycetidae. What other names have been given to this sub-class?
9. Characterize the Tremellales.
10. Diagram the probable life cycle of Dacrymyces deliquescens.
11. Describe the probable evolution of the basidiocarp in the Dacrymycetaceae citing specific genera to illustrate your discussion.
12. Describe the formation, dispersal, and germination of the oidia in Dacrymyces.
13. Describe the germination of the basidiospores in Dacrymyces.

D. TEXT REFERENCES:

Alexopoulos -- pp. 426-442, 443-446.
Bessey -- pp. 449-451.

E. SELECTED REFERENCES:

1. BARNETT, H. L. 1937. Studies in the sexuality of the Heterobasidiae. Mycologia, 29:626-649.
2. BODMAN, M. C. 1938. Morphology and cytology of Guepinia spathularia. Mycologia, 30:635-652.
3. BULAT, T. J. 1953. Cultural studies of Dacrymyces ellisii. Mycologia, 45:40-45.
4. BULAT, T. J. 1954. Effect of light on color in Dacrymyces. Mycologia, 46:32-36.
5. BULLER, A. H. R. 1922. Researches on fungi. Vol. II. xii + 492 pp. 157 figs. Longmans, Green, and Co., London.
6. DONK, M. A. 1954. A note on sterigmata in general. Bothalia, 6:301-302.
7. GILBERT, E. M. 1921. Cytological studies of the lower Basidiomycetes. I. Dacrymyces. Trans. Wis. Acad. Sci., Arts, Lett., 20:387-397.
8. KENNEDY, L. L. 1958a. The genera of the Dacrymycetaceae. Mycologia, 50:874-895.
9. KENNEDY, L. L. 1958b. The genus Dacrymyces. Mycologia, 50:896-915.
10. LINDER, D. H. 1940. Evolution of the Basidiomycetes and its relation to the terminology of the basidium. Mycologia, 32:419-447.
11. MARTIN, G. W. 1952. Revision of the North Central Tremellales. State University Iowa St. Nat. Hist., 19, #3, 122 pp. 38 figs.
12. MARTIN, G. W. 1961. Key to the families of fungi. In Dictionary of the fungi, pp. 497-517. G. C. Ainsworth. Commonwealth Mycological Institute, Kew, Surrey.
13. ROGERS, D. P. 1934. The basidium. Univ. Iowa Stud. Nat. Hist., 16:160-183.
14. SAVILE, D. B. O. 1955. A phylogeny of the Basidiomycetes. Can. Jr. Bot., 33:60-104.
15. TALBOT, P. H. B. 1954. Micromorphology of the lower hymenomycetes. Bothalia, 6:249-299.
16. YEN, H. C. 1949. Contributions a l'étude de la sexualité et du mycelium des Basidiomycètes saprophytes. Ann. l'univ. Lyon, Sect. C, Sci, nat., 6:1-158.

F. GLOSSARY:

1. Basidiocarp (Gr. basidion = small base, basidium + karpos = fruit): a fruiting body which bears basidia.
2. Epibasidium (pl. epibasidia; Gr. epi = upon + basidion = a small base): the upper portion of the basidial apparatus in the Heterobasidiomycetidae.

3. Hypobasidium (pl. hypobasidia; Gr. hypo= under + basidion = a small base): the basal portion of the basidial apparatus in the Heterobasidiomycetidae.
4. Probasidium (pl. probasidia; Gr. pro = before + basidion = a small base): a binucleate hyphal cell which develops into a basidium, usually after undergoing some morphological changes.
5. Promycelium (pl. promycelia; Gr. pro = before + mycelium): the epibasidium of the rusts, smuts. A germ tube issuing from the teleutospore, which bears the basidiospores.
6. Teleutospore (Gr. teleutaios = last + sporos = seed, spore): a thick-walled resting spore of some Heterobasidiomycetidae, notably the rusts and the smuts, in which karyogamy occurs; it is a part of the basidial apparatus.

Class Basidiomycetes – Sub-Class Heterobasidiomycetidae

Order Tremellales (Cont.)

Family: Tremellaceae

Genera: Exidia, Tremella, Phlogiotis

A. GENERAL REMARKS:

1. The Tremellaceae include those jelly fungi which bear their basidiospores on longitudinally or obliquely septate basidia.

B. PROCEDURE:

2. Examine the basidiocarps of as many genera as are made available to you and note the variation from simple to complex structure.

3. Mount a portion of the basidiocarp of Exidia or Tremella sp. in lacto-phenol containing cotton blue. Search for the top-shaped hypobasidia bearing long epibasidia. Pay particular attention to the longitudinal septa dividing the basidium into four quarters. Find the basidiospores and if possible note method of attachment to the epibasidia.

4. Examine the basidiocarps of Phlogiotis. Locate the hymenium.

C. QUESTIONS:

1. How do the Tremellaceae differ from the Dacrymycetaceae?
2. How does dikaryotization take place in Exidia?
3. Describe the development of the basidium in Exidia.
4. Describe the germination of the basidiospores in Exidia.
5. Are the Tremellaceae of any economic importance?
6. How do the Sirobasidiaceae differ from the Tremellaceae?
7. Describe the basidium of the Tulasnellaceae.

D. TEXT REFERENCES:

Alexopoulos -- pp. 446-449.
Bessey -- pp. 451-455.

E. SELECTED REFERENCES:

1. BANDONI, R. J. 1956. A preliminary survey of the genus Platygloea. Mycologia, 48:821-840.
2. BARNETT, H. L. 1937. Studies in the sexuality of the Heterobasidiae. Mycologia, 29:626-649.
3. KAO, C. J. 1956. The cytology of Xenogloea eriophori. Mycologia, 48:288-301.
4. LINDER, D. H. 1940. Evolution of the Basidiomycetes and its relation to the terminology of the basidium. Mycologia, 32:419-447.
5. MARTIN, G. W. 1944. The Tremellales of the North Central United States and adjacent Canada. Univ. Iowa Stud. Nat. Hist., Vol. 18, No. 3. 88 pp.
6. MARTIN, G. W. 1952. Revision of the North Central Tremellales. State Univ. Iowa Stud. Nat. Hist., 19, #3, 122 pp. 38 figs.
7. MARTIN, G. W. 1957. The tulasnelloid fungi and their bearing on basidial terminology. Brittonia, 9:25-30.
8. OLIVE, L. S. 1957. Two new genera of the Ceratobasidiaceae and their phylogenetic significance. Am. Jr. Bot., 44:429-435.
9. ROGERS, D. P. 1932. A cytological study of Tulasnella. Bot. Gaz., 94:86-105.

10. ROGERS, D. P. 1933. Taxonomic review of the Tulasnellaceae. Ann. Mycol. 31:181-203.
11. ROGERS, D. P. 1936. Basidial proliferation through clamp formation in a new Sebacina. Mycologia, 28:347-362.
12. TEIXEIRA, A. R. and D. P. ROGERS. 1955. Aporpium, a polyporoid genus of the Tremellaceae. Mycologia, 47:408-415.
13. WELLS, K. 1957. Studies of some Tremellaceae. Lloydia, 20:43-65.
14. WELLS, K. 1958. Studies of some Tremellaceae. II. The genus Ductifera. Mycologia, 50:407-416.
15. WELLS, K. 1959. Studies of some Tremellaceae. III. The genus Bourdotia. Mycologia, 51:541-563.
16. WELLS, K. 1961. (1962). Studies on some Tremellaceae. IV. Exidiopsis. Mycologia, 53:317-370.
17. WHELDEN, R. M. 1934. Cytological studies in the Tremellaceae. I. Tremella. Mycologia, 26:415-435.
18. WHELDEN, R. M. 1935. II. Exidia. Mycologia, 27:41-57.
19. WHELDEN, R. M. 1935. III. Sebacina. Mycologia, 27:503-520.
20. WHELDEN, R. M. 1937. IV. Protodontia and Tremellodendron. Mycologia, 29:100-115.

F. GLOSSARY:

1. Dikaryotization (Gr. Dis = twice + karyon = nut, nucleus): the process of rendering a cell binucleate; usually accomplished by plasmogamy.

Class Basidiomycetes–Sub-Class Heterobasidiomycetidae

Order Tremellales (Cont.)

Family: Auriculariaceae

Genus: Auricularia

A. GENERAL REMARKS:

1. The Auriculariaceae are jelly fungi which bear their basidiospores on transversely septate basidia. The basidiocarps of some genera are gelatinous, but those of others may be waxy or cartilaginous. In plant parasitic species the basidiocarp may be inconspicuous or absent.

B. PROCEDURE:

2. Examine the fructification of Auricularia auricularis. Note the color, size, and various folds of the surface.

3. Mount a portion of the hymenial layer of the basidiocarp and study the transversely septate hypobasidia, the epibasidia, the sterigmata, and the basidiospores.

Genus: Herpobasidium

4. Examine honeysuckle leaves which are parasitized by Herpobasidium deformans. Look for the curved, septate basidia which issue from the stomata of the leaves.

5. Study the conidial stage of the fungus. Note the globose conidia borne in heads on the branched conidiophores.

Family: Phleogenaceae

6. The basidiocarps of the Phleogenaceae are angiocarpous or hemiangiocarpous; they may be gelatinous or dry.

Genus: Phleogena

7. Using a binocular dissecting microscope examine the hemiangiocarpous fructifications of Phleogena faginea growing on beech bark. Note the stalk and round head of each fructification. Observe the peridium-like covering of the head.

8. Mount a small portion of the powdery interior of the head. Observe the large number of basidiospores borne on the basidia. Note that the basidiospores are sessile, without sterigmata. Look for clamp connections on the hyphae below the basidia.

Family: Septobasidiaceae

9. The Septobasidiaceae are parasitic on scale insects. The basidiocarps are dry. The probasidia are often thick-walled cysts.

Genus: Septobasidium

10. Study any species of Septobasidium which may be available. Note the elaborate basidiocarps formed.

11. Locate the hymenial layer and mount some teleutospores for study. Note the thick walls.

C. QUESTIONS:

1. Discuss the economic importance of the Auriculariaceae.
2. Discuss the biological relationship of the Septobasidiaceae with their insect hosts.
3. Of what evolutionary significance may the probasidium of the Septobasidiaceae be?
4. Of what particular mycological interest is the Family Ceratobasidiaceae?
5. From your study of the foregoing orders and the reading of some key references, discuss the evolution of the Tremellales.
6. Discuss the taxonomy fo the jelly fungi as presented by Martin (1945), Linder (1940), Gäumann (1949), and Bessey (1950).

D. TEXT REFERENCES:

Alexopoulos -- pp. 449-453.
Bessey -- pp. 438-449.

E. SELECTED REFERENCES:

1. BARNETT, H. L. 1937. Studies in the sexuality of the Heterobasidiae. Mycologia, 29:626-649.
2. BOEDJIN, K. B. 1959. A species of Septobasidium shedding its immature basidia. Persoonia, 1:21-23.
3. COUCH, J. N. 1937. A new fungus intermediate between the rusts and Septobasidium. Mycologia, 29:665-673.
4. COUCH, J. N. 1938. The genus Septobasidium. ix + 480 pp. 60 text figs. 114 pls. Frontis. University of North Carolina Press. Chapel Hill.
5. GOULD, C. J., JR. 1945. The parasitism of Glomerularia lonicerae (Pk.) D. of H. in Lonicera sp. Iowa St. College. Jour. Sci. 19:301-331.
6. GÄUMANN, E. A. 1949. The Fungi. (English Translation by F. L. Wynd, 1952.) Hafner Publishing Co., New York.
7. LINDER, D. H. 1940. Evolution of the Basidiomycetes and its relation to the terminology of the basidium. Mycologia, 32:419-447.
8. LOWY, B. 1951. A morphological basis for classifying the species of Auricularia. Mycologia, 43:351-358.
9. LOWY, B. 1952. The genus Auricularia. Mycologia, 44:656-692.
10. MARTIN, G. W. 1944. The Tremellales of the North Central United States and adjacent Canada. Univ. Iowa Stud. Nat. Hist, Vol. 18, No. 3. 88 pp.
11. MARTIN, G. W. 1945. The classification of the Tremellales. Mycologia, 37:527-542.
12. MARTIN, G. W. 1952. Revision of the North Central Tremellales. State Univ. Iowa Stud. Nat. Hist., 19, #3, 122 pp. 38 figs.
13. SHEAR, C. L., and DODGE, B. O. 1925. The life history of Pilacre faginea (fr.) B. and Br. Jour. Agr. Res., 30:407-417.
14. STANLEY, I. N. 1940. Development of the basidium of Eocronartium muscicola. Trans. Am. Micr. Soc., 59:407-413.

Class Basidiomycetes – Sub-Class Heterobasidiomycetidae

Order: Uredinales

A. GENERAL REMARKS:

1. The Uredinales are the plant rusts. They are specialized parasites with a very complex life cycle. The basidial apparatus of the rusts is represented by the teleutospore (a probasidial cyst behaving as a hypobasidium) and the slender, four-celled promycelium (epibasidium) which issues from it upon germination. Each cell of the promycelium produces a sterigma upon which a basidiospore is formed.

2. Rust fungi are either long-cycled (macrocyclic) or short-cycled (microcyclic). A typical long-cycled rust passes through the following reproductive phases in the order given.

   Stage O Spermogonia bearing spermatia and receptive hyphae.
   Stage I Aecia bearing aeciospores.
   Stage II Uredia bearing uredospores.
   Stage III Telia bearing teleutospores.
   Stage IV Promycelia bearing basidiospores.

   Aeciospores, uredospores and teleutospores are binucleate. Basidiospores, spermatia, and receptive hyphae are uninucleate. Karyogamy takes place in the teleutospore and meiosis in the promycelium.

3. The teleutospores which are considered to represent the perfect stage of the rusts may be free, loosely arranged, or fused into a compound head, a crust, or a column.

4. The Uredinales are classified into three families: the Pucciniaceae, Melampsoraceae and Coleosporiaceae.

B. PROCEDURE:

Family: Pucciniaceae

5. The teleutospores of the Pucciniaceae are borne free from each other, fascicled on a stalk, or embedded in a gelatinous matrix.

Genus: Puccinia

6. Make a study of Puccinia graminis as follows:

   a. Stages O and I: Examine barberry leaves infected with the rust under the dissecting microscope. Note the tiny spermogonia on the upper epidermis. Invert the leaf and observe the cluster cups (aecia) which have pushed through the lower epidermis. Note particularly the lip of the aecium. This type of aecium typifies the imperfect rust genus Aecidium.

CLASS BASIDIOMYCETES

VARIOUS TYPES OF TELEUTOSPORES

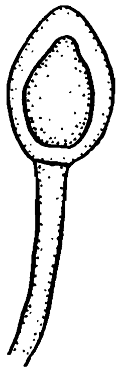


FIG. 68

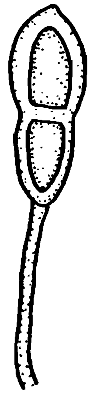


FIG. 69

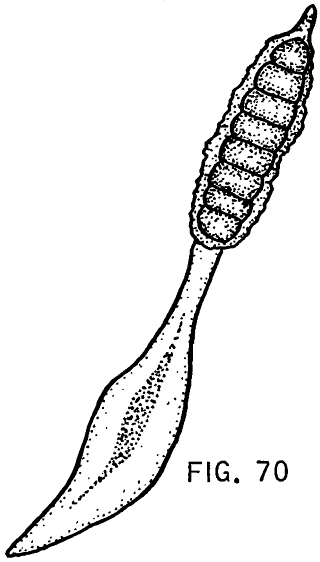


FIG. 70

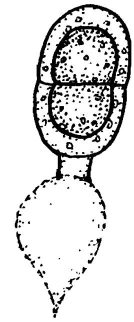


FIG. 71

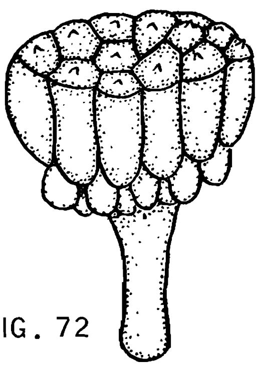


FIG. 72

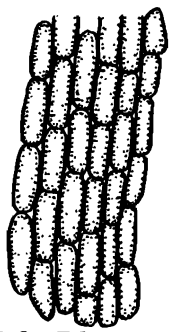


FIG. 73

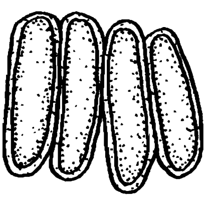


FIG. 74

FIGS. 68-74 TELEUTOSPORES OF VARIOUS RUSTS (UREDINALES)

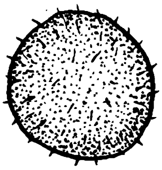


FIG. 75

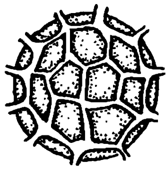


FIG. 76

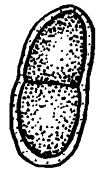


FIG. 77

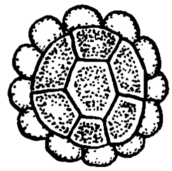


FIG. 78

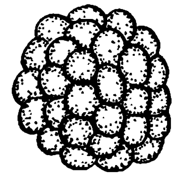


FIG. 79

FIGS. 75-79 TELEUTOSPORES OF VARIOUS SMUTS (USTILAGINALES)

Plate 15

b. Study prepared slides with stained cross-sections of infected barberry leaves and note the position of spermogonia and aecia with relation to the leaf tissues.

c. Study the spermogonia under the oil immersion objective. Note the spermatiophores, spermatia and periphyses. Some sections also show the drops of nectar exuding from the spermogonia.

d. Now study a mature aecium. Note the basal "aeciospore mother cells" from which the chains of aeciospores and the peridium arise. Note that near the base of the aecium the spore chains consist of alternately formed aeciospores and disjunctor cells.

e. Stage II. Examine infected wheat stems and find the "summer stage" of the fungus. Note the rusty color of the pustules which is responsible for the term "rust" being applied to these fungi. The summer sorus is the uredium which produces a large number of uredospores. Uredospores function as conidia in that they are able to re-infect the wheat and produce more uredia.

f. Scrape a uredium gently with a scalpel or needle and mount some uredospores onto a slide in lacto-phenol. Study under the oil immersion objective. Note the color of the spores, their shape, method of attachment. Study the wall, its thickness, its minute spines and the two lateral germ pores from which the germ tubes will push out during germination.

g. Study a prepared slide showing cross-sections of a wheat stem bearing uredia. Observe origin of the uredium and its effect on the host tissues, particularly the epidermis. Note the long stalks of the uredospores.

h. Stage III. Observe wheat straw which bears the telial stage of the fungus. Note the black pustules which give the disease its name "black stem rust of wheat". Study the teleutospores in a lacto-phenol mount under the high power and oil immersion objective. Note their color, shape, number of cells, and thickness of the wall.

i. Study prepared slides showing cross-sections of a wheat stem bearing telia. Note the manner in which teleutospores are borne and their position with reference to the host cells and to each other.

7. Study the teleutospores of other species of *Puccinia*, such as *Puccinia malvacearum*, and *Puccinia asparagi*, and note the difference in the shape of the spores.

Genus: Gymnosporangium

8. The genus *Gymnosporangium* differs from *Puccinia* in that the teleutospores have a very much longer stalk and are embedded in a gelatinous substance. *Gymnosporangium juniperi-virginianae* or *G. globosum* cause cedar and apple rust.

9. Observe the galls or "cedar apples" on the twigs of *Juniperus*, and note the extended telia. Study the two-celled teleutospores from "cedar-apple" by mounting a portion of the gelatinous telium in water or lacto-phenol.

10. Study the aecia of *G. juniperi-virginianae* growing on the under side of apple leaves. Using a dissecting microscope, observe how the lip of the peridium is split and turned outward forming a characteristic fringe. Contrast with the aecia of *G. globosum* on hawthorn leaves. The aecia of *Gymnosporangium* typify the imperfect rust genus *Roestelia*.

11. Study prepared slides showing cross-sections of apple leaves bearing spermogonia and aecia of *G. juniperi-virginianae*.

Genera: Uromyces, Phragmidium, Nyssopsora, Uropyxis, Ravenelia

12. Study the teleutospores of the genera listed above and any others available and contrast with those of *Puccinia* and *Gymnosporangium*.

13. Study the aecia of any species of *Phragmidium*. Note the absence of a peridium. This type of aecium typifies the imperfect rust genus *Caeoma*.

Family: Melampsoraceae

14. The Melampsoraceae produce their teleutospores united laterally into crusts or columns.

Genus: Cronartium

15. *Cronartium ribicola* is the cause of white pine blister rust. Study the various stages of this fungus as follows:

    a. Examine infected currant and gooseberry leaves under the binocular dissecting microscope and note the long columnar telia and the small dome-shaped uredia.

    b. Study prepared slides showing cross-sections of the telia and note how the teleutospores are arranged.

    c. Examine white pine stems showing the symptoms of blister rust. The white blister-like structures are the aecia of the fungus. Note the prominent peridium characteristic of the imperfect rust genus *Peridermium* to which this aecial stage belongs.

    d. Study prepared slides of cross-sections of infected white pine stems. Locate a spermogonium. Contrast with the spermogonia of *Puccinia* and *Gymnosporangium*. Locate an aecium and note its position with reference to the spermogonia.

Family: Coleosporiaceae

16. The Coleosporiaceae produce their teleutospores united laterally into layers or crusts. Upon germination the teleutospores become 3-septate (4-celled) instead of producing a promycelium, and each cell gives rise to a basidiospore on a sterigma.

Genus: Coleosporium

17. Coleosporium solidaginis produces Stages O and I on pine needles and Stages II and III on goldenrod (Solidago). Study the various stages. Pay particular attention to the uredospores. These are produced in chains in the manner of aeciospores.

C. QUESTIONS:

1. Explain how the rusts obtain nourishment from their hosts.
2. What are autoecious rusts? Heteroecious rusts?
3. How do long-cycle rusts differ from short-cycled rusts?
4. How do the rusts reproduce sexually?
5. Why are the uredospores considered to be the conidia of the rusts?
6. What structures represent the basidia of the rusts? Explain.
7. Will the eradication of all the barberry from the United States and Canada effect complete control of wheat rust? Explain.
8. Discuss physiological specialization in Puccinia graminis.
9. How is barberry eradication related to the problem of a wheat breeding program aimed at the production of rust resistant wheat varieties?
10. Compare the effectiveness of barberry eradication and currant eradication in controlling wheat rust and white pine blister rust respectively, and give reasons for your answer.
11. What is a "cedar apple"? What are the gelatinous "horns"?
12. Construct a key to the families of the Uredinales.
13. Construct a detailed life-cycle diagram of Puccinia graminis. Divide the cycle into haploid, dikaryotic and diploid portions. Indicate the barberry phases and the grass phases of the cycle.

D. TEXT REFERENCES:

Alexopoulos -- pp. 455-477.
Bessey -- pp. 382-409.

E. SELECTED REFERENCES:

1. ALLEN, RUTH F. 1930. A cytological study of heterothallism in Puccinia graminis. Jour. Agr. Res. 40:585-614.
2. ALLEN, RUTH F. 1933. Further cytological studies of heterothallism in Puccinia graminis. Jour. Agr. Res. 47:1-16.
3. ARTHUR, J. C. 1934. Manual of the rusts of the United States and Canada. xv + 438 pp. 487 figs. Purdue Research Foundation. Lafayette.
4. BAXTER, J. W. 1959. A monograph of the genus Uropyxis. Mycologia, 51:210-226.
5. BULLER, A. H. R. 1938. Fusion between flexuous hyphae and pycnidiospores in Puccinia graminis. Nature 141:33-34.
6. BULLER, A. H. R. 1950. Researches on fungi. Vol. VII. xx + 458 pp. 124 figs. University of Toronto Press. Toronto.
7. CHESTER, K. S. 1946. The cereal rusts. xvi + 269 pp. 11 figs. Frontisp. Chronica Botanica Co., Waltham, Mass.
8. CRAIGIE, J. H. 1927. Discovery of the funtion of the pycnia of the rust fungi. Nature 120:765-767.
9. CRAIGIE, J. H. 1931. An experimental investigation of sex in the rust fungi. Phytopath. 21:1001-1040.
10. CRAIGIE, J. H. 1959. Nuclear behavior in the diploidization of the haploid infections of Puccinia helianthi. Can. Jr. Bot., 37:843-855.
11. CUMMINS, G. B. 1956. Host index and morphological characterization of the grass rusts of the world. Plant Dis. Rptr., Suppl. 237. U. S. Dept. Agr., Washington.
12. CUMMINS, G. B. 1959. Illustrated genera of rust fungi. ii + 131 pp. Illustr. Burgess Publishing Co., Minneapolis.
13. CUTTER, V. M., JR., 1959 (1961). Studies on the isolation and growth of plant rusts in host tissue culture and upon synthetic media. I. Gymnosporangium. Mycologia, 51:248-295.
14. CUTTER, V. M. 1960 (1961). Studies on the isolation and growth of plant rusts in host tissue culture and upon synthetic media. II. Uromyces ari-triphylli. Mycologia, 52:726-742.

15. GROVE, W. R. 1913. The British rust fungi (Uredinales). Their biology and classification. xi + 412 pp. 290 figs. Cambridge University Press. Cambridge.
16. HOTSON, H. H., and V. M. CUTTER, JR. 1951. The isolation and culture of Gymnosporangium juniperi-virginianae Schw. upon artificial media. Proc. Nat. Acad. Sci. U. S. 37:400-403.
17. HUNTER, LILLIAN M. 1948. A study of the mycelium and haustoria of the rusts of Abies. Can. Jr. Res., C26: 219-238.
18. LINDER, D. H. 1940. Evolution of the Basidiomycetes and its relation to the terminology of the basidium. Mycologia, 32:419-447.
19. MARTIN, G. W. 1938. The morphology of the basidium. Am. Jour. Bot., 25:682-685.
20. MC ALPINE, D. 1906. The rusts of Australia. vii + 349 pp. 28 figs. 55 pls. Dept. Agr. Victoria. Melbourne.
21. OLIVE, L. S. 1947. Cytology of the teliospores, basidia, and basidiospores of Aphenospora kevorkiana Linder. Mycologia, 39:409-425.
22. OLIVE, L. S. 1949. Karyogamy and meiosis in the rust Coleosporium vernoniae. Am. Jour. Bot. 36:41-54.
23. PAYAK, M. M. 1956. A study of the pycnia, flexuous hyphae, and nuclear migration in the aecia of Scopella gentilis. Bot. Gaz., 118:37-42.
24. SAVILE, D. B. O. 1954a. Cellular mechanics, taxonomy, and evolution in the Uredinales and Ustilaginales. Mycologia, 46:736-761.
25. SAVILLE, D. B. O. 1954b. Taxonomy, phylogeny, host relationship and phytogeography of the microcyclic rusts of Saxifragaceae. Can. Jr. Bot., 32:400-425.
26. SAVULESCU, T. 1953. Monografia uredinalelor din republica populara Romana. 2 vols. 1166 pp. + Appendix I-XX to Vol. I. 948 figs. 54 pls. (6 col.).
27. WALKER, J. C. 1950. Plant Pathology. x + 699 pp. 194 figs. McGraw-Hill Book Co., Inc. New York.

F. GLOSSARY:

1. Aeciospore (Gr. aikia = injury + sporos = seed, spore): a binucleate spore produced in an aecium.
2. Aecium (pl. aecia; Gr. aikia = injury): a group of binucleate cells which give rise to spore chains by successive, conjugate division of their nuclei.
3. Telium (pl. telia; Gr. telos = end): a group of binucleate cells which produce teleutospores.
4. Uredium (pl. uredia; L. urere = to burn): a group of binucleate cells which produce uredospores.
5. Uredospore (L. urere = to burn + Gr. sporos = seed, spore): a binucleate repeating spore, of the Uredinales.

Class Basidiomycetes
Sub-Class Heterobasidiomycetidae

Order: Ustilaginales

A. GENERAL REMARKS:

1. The Ustilaginales are the smuts. Like the rusts, they produce their basidiospores on a promycelium which grows out of a germinating teleutospore.

2. In contrast to the rusts, the smuts (a) produce their teleutospores from intercalary hyphal cells somewhat in the manner of chlamydospores, (b) they bear their basidiospores not on sterigmata but sessile, (c) they do not discharge their basidiospores violently, and (d) they produce no specialized sex organs.

3. The Ustilaginales are divided into three families: the _Ustilaginaceae_, the _Tilletiaceae_, and the _Graphiolaceae_. We shall consider only two of these.

B. PROCEDURE:

Family: Ustilaginaceae

Genus: Ustilago

4. The teleutospores of the Ustilaginaceae produce a 3-septate (4-celled) promycelium from which the basidiospores are budded off laterally.

5. Examine various parts of a corn plant infected with _Ustilago maydis_ (_U. zeae_). Note that the smut balls may be produced anywhere on the plant above the ground.

6. Mount some smut spores (teleutospores) and study under the oil immersion objective. Note the minute spines on the surface of the spores, the thick walls, the color, and the size of the spores.

7. Make a hanging drop of some teleutospores in distilled water and, at the next laboratory period, study the germination of the spores.

8. Study the teleutospores of _Ustilago tritici_ and of other species of _Ustilago_ and note how they differ in their size and markings.

Genera: Sorosporium, Schizonella, Sphacelotheca, Cintractia, Tolyposporium

9. Study the teleutospores of as many of the above genera as are available and contrast with _Ustilago_. Note particularly the spore groupings which are characteristic of these genera and the presence or absence of a membrane around the smut balls.

Family: Tilletiaceae:

Genus: Tilletia

10. The teleutospores of the Tilletiaceae produce a non-septate promycelium which bears a cluster of basidiospores at the tip.

11. Examine heads of wheat infected with Tilletia caries. Contrast their appearance with that of wheat heads infected with Ustilago tritici.

12. Study the teleutospores of Tilletia caries, and note their size, color, thickness of wall and surface markings.

13. Study the teleutospores of other species of Tilletia.

14. Prepare a hanging drop of teleutospores of T. caries in distilled water and examine at the next laboratory period for germination. Contrast with Ustilago maydis.

Genera: Entyloma, Urocystis, Tuburcinia, Doassansia

15. Study the teleutospores of as many of the above genera as are available and learn to recognize them. Note the characteristic groupings of cells and the differentiation of some spore balls into fertile and sterile regions.

C. QUESTIONS:

1. In tabular form compare and contrast the Uredinales and the Ustilaginales.
2. Distinguish between the three families of the Ustilaginales.
3. What are loose smuts? Covered smuts?
4. Explain in some detail the method of teleutospore formation in the Ustilaginales.
5. Explain tetrapolarity in the smuts.
6. Construct a detailed life cycle diagram for Ustilago maydis and one for Tilletia caries.

D. TEXT REFERENCES:

Alexopoulos -- pp. 477-486.
Bessey -- pp. 409-424.

E. SELECTED REFERENCES:

1. AINSWORTH, G. C. and KATHLEEN SAMPSON. 1950. The British smut fungi. 137 pp. 21 figs. Commonwealth Mycological Institute, Kew, Surrey.
2. CLINTON, G. P. 1904. North American Ustilagineae. Proc. Boston Soc. Nat. Hist. 31:329-529.
3. FISCHER, G. W. 1951. The smut fungi. A guide to the literature with bibliography. x + 387 pp. The Ronald Press Co. New York.
4. FISCHER, G. W. 1953. Manual of North American smut fungi. xii + 343 pp. 136 figs. The Ronald Press Co., New York.
5. FISCHER, G. W. and C. S. HOLTON. 1957. Biology and control of the smut fungi. x + 622 pp. 107 figs. Frontisp. The Ronald Press Co., New York.
6. HANNA, W. F. 1929. Studies in the physiology and cytology of Ustilago zeae and Sorosporium reilianum. Phytopath. 19:415-442.
7. HOLTON, C. S., and F. D. HEALD. 1941. Bunt or stinking smut of wheat. ii + 211 pp. 21 figs. Planographed. Burgess Publishing Co. Minneapolis.
8. HOLTON, C. S. and E. L. KENDRICK. 1957. Fusion between secondary sporidia in culture as a valid index of of sex compatibility in Tilletia caries. Phytopath., 47:688-689.
9. KENDRICK, E. L. 1957. The production of teliospores of Tilletia caries in culture. Phytopath., 47:674-676.

10. SAVULESCU, T. 1957. Ustilaginalele din republica populara Romana. 2 vols. 1168 pp. 594 figs. 21 col. pls.
11. THIRUMALACHAR, M. J., and J. G. DICKSON. 1947. Chlamydospore germination and artificial culture of Ustilago striiformis from timothy and bluegrass. Phytopath. 37:730-734.
12. THIRUMALACHAR, M. J. and J. G. DICKSON. 1949. Chlamydospore germination, nuclear cycle and artificial culture of Urocystis agropyri on red top. Phytopath. 39:333-339.
13. ZUNDEL, G. L. 1953. The Ustilaginales of the world. Penn. State Univ. Contrib. 176. xi + 410 pp. 3 figs.

CLASS BASIDIOMYCETES — FRUITING BODIES

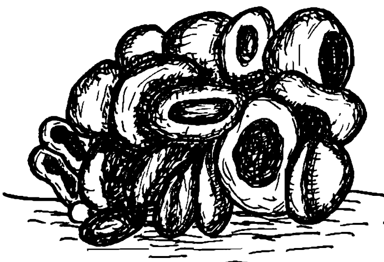


FIG. 80 JELLY FUNGUS

FIG. 81 JELLY FUNGUS

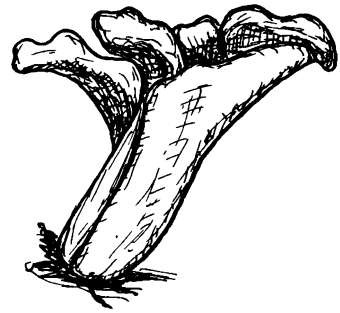


FIG. 82 JELLY FUNGUS

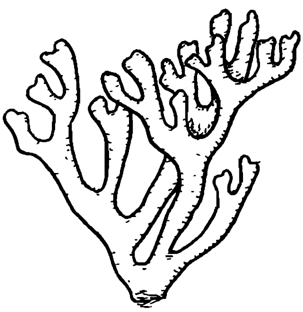


FIG. 83 CORAL FUNGUS

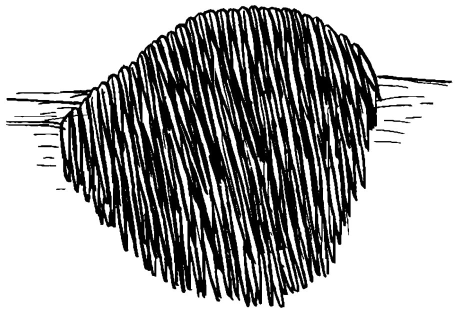


FIG. 84 TOOTH FUNGUS

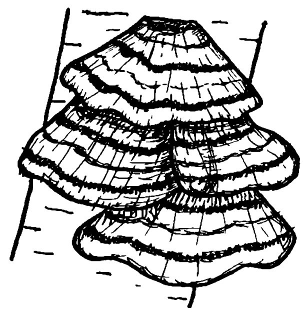


FIG. 85 BRACKET FUNGUS

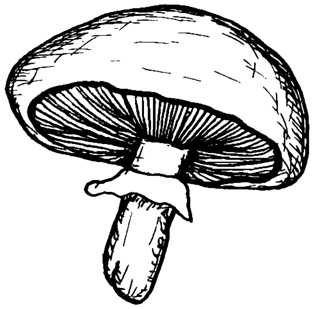


FIG. 86 MUSHROOM

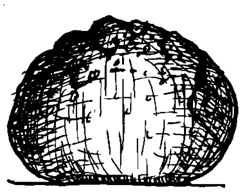


FIG. 87 PUFFBALL

FIG. 88 EARTHSTAR

FIG. 89 STALKED PUFFBALL

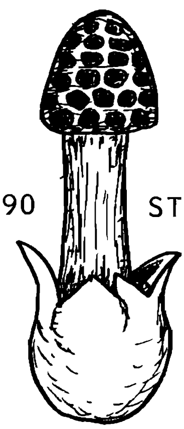


FIG. 90 STINKHORN

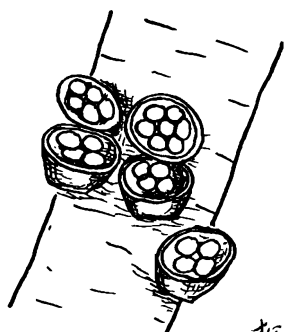


FIG. 91 BIRDS' NEST FUNGUS

Plate 16

Class Basidiomycetes – Sub-Class Homobasidiomycetidae

A. GENERAL REMARKS:

1. The Homobasidiomycetidae bear their basidiospores on simple, club-shaped basidia produced terminally on the hyphae. Such a basidium is termed a holobasidium.

2. With relatively few exceptions, these fungi produce their basidia in well-developed fruiting bodies, the basidiocarps.

3. Most Homobasidiomycetidae fall into two large groups or series: the Hymenomycetes and the Gasteromycetes. The former produce their basidia in well formed hymenial layers and expose the hymenia before the basidiospores mature; the latter (Gasteromycetes) produce their basidia in closed basidiocarps and either do not expose them at all, or expose their spores only after the spores are mature.

4. In addition to these two series, there is a small order Exobasidiales whose relationships are obscure .

B. PROCEDURE:

Order: Exobasidiales

Family: Exobasidiaceae

Genus: Exobasidium

5. Examine young twigs and leaves of Vaccinium sp. which are infected with Exobasidium vaccinii. Note the reddish color and the swollen tissues characteristic of the infected parts.

6. Prepare a very thin free-hand section through the infected tissues and examine in a water or lacto-phenol mount. Note that the basidia form a surface layer (hymenium) reminiscent of the hymenium of Taphrina.

Series: Hymenomycetes

7. The Hymenomycetes are classified in two orders: Polyporales and Agaricales.

Order: Polyporales

8. The Polyporales bear their basidia in various ways. The hymenium may be smooth, ridged, warty, or spiny, or it may line the inside surface of tubes or pores, or the external surface of gills. If tubes, pores, or gills are present the basidiocarp is not soft and fleshy at maturity.

9. The Polyporales are subdivided into many families, the number varying with the author. We shall study representatives of four families.

Family: Thelephoraceae

10. The Thelephoraceae are a heterogeneous family which modern taxonomists are splitting into more homogeneous and therefore more natural smaller units. More work needs to be done before we have a clear idea on the number of families which will emerge, but much progress is being made.

11. The hymenium may be smooth, roughened, or corrugated and the basidiocarps may be cobwebby, membranous, leathery, or hard.

Genera: Corticium, Tomentella, Stereum

12. Examine dead twigs and branches of trees bearing the thin, basidiocarps of Corticium and related genera. The basidiocarp here is represented by a thin, cottony, or felty mass of hyphae bearing basidia. Study prepared, stained slides showing the structural features.

13. Study a number of species of Stereum. Note the papery, leathery or woody basidiocarps, bearing a smooth or wrinkled hymenium on one side, characteristic of this and related genera.

Family: Clavariaceae

Genera: Clavaria, Clavicorona, Clavulinopsis, Ramaria, Clavariadelphus

14. Study the club-shaped and coral-shaped, erect basidiocarps of Clavaria and related genera. Locate the hymenial layer in each case. If fresh material is available, cut thin cross-sections through the hymenium-bearing portion of a basidiocarp and note the arrangement of the basidia.

Family: Hydnaceae

Genera: Hydnum, Hericium, Steccherinum, Echinodontium

15. Examine fresh, dried, or preserved specimens of Hericium, Steccherinum, and any other genera available, and familiarize yourself with the various types of basidiocarps. Note that the teeth which bear the hymenia are pendent in contrast to the erect hymenium-bearing branches of the Clavariaceae.

16. Study prepared stained slides through a fertile region of a basidiocarp and note the arrangement of the basidia. Locate the trama and the hymenium.

17. If fresh material is available, identify as many species as you can using an appropriate key.

Family: Polyporaceae

Genera: Polyporus, Fomes, Trametes, Ganoderma, Favolus, Lenzites, Daedalia, Irpex.

18. The hymenia of the Polyporaceae line the inner walls of pores or tubes of various types of basidiocarps. Study the basidiocarps of representative genera of the Polyporaceae and learn to distinguish among them.

19. Note that the "pores" of Lenzites and Daedalia are so gill-like that many authors include these two genera in keys to the Agaricaceae (gill-fungi) as well as keys to the Polyporaceae.

20. Study prepared, stained slides of cross sections through the hymenial layers and note the arrangement of the basidia. Locate the trama and hymenium.

21. The genus Irpex is intermediate between the Polyporaceae and the Hydnaceae. Study the surface of the basidiocarp under the high power of the dissecting microscope and note that the "teeth" appear to be the irregular jagged walls of poroid or tubular structures.

22. Using an appropriate key identify as many species of Polyporaceae as time and material permit.

C. QUESTIONS:

1. Compare and contrast the two sub-classes of the Basidiomycetes.
2. Characterize the Exobasidiales. Discuss the various viewpoints on the origin and relationships of these fungi.
3. Characterize the Polyporales. Construct a simple key to the families of the Polyporales usually recognized.
4. What families have been split off the Thelephoraceae by modern taxonomists?
5. Discuss the use of hyphal structure as an important character in the taxonomy of the Polyporales (see Corner, 1932a, 1932b, 1950).
6. How may cultural characters be used to differentiate among taxa in the Polyporales?
7. What is the economic importance of the Polyporales?

D. TEXT REFERENCES:

Alexopoulos -- pp. 492-504.
Bessey -- pp. 464-495.

E. SELECTED REFERENCES:

1. BADCOCK, E. C. 1943. Methods of obtaining fructifications of wood-rotting fungi in culture. Trans. Brit. Mycol. Soc., 26:127-132.
2. BULLER, A. H. R. 1941. The diploid cell and the diploidization process in plants and animals with special reference to the higher fungi. Bot. Rev. 7:335-387, 389-431.
3. BRODIE, H. J. 1951. The function of the cups of Polyporus conchifer. Science, 114:636.
4. BURT, A. 1914-1926. The Thelephoraceae of North America. Parts I - XV. In Ann. Mo. Bot. Gard., Vols. 1-7 and 11-13.
5. CHARLES, VERA K. 1946. Some common mushrooms and how to know them. U. S. Dept. of Agr., Circular No. 143. Supt. of Documents. U. S. Gov. Printing Office, Washington, D. C.
6. COKER, W. C. 1923. The Clavarias of the United States and Canada. 209 pp. 92 pls. Univ. of North Carolina Press. Chapel Hill.
7. COKER, W. C. and ALMA BEERS. 1951. The stipitate hydnums of the Eastern United States and Canada. ix + 201 pp. 123 pls.
8. COOKE, W. B. 1957. The Porotheleaceae: Porotheleum. Mycologia, 49:680-693.
9. COOKE, W. B. 1959. The genera of pore fungi. Lloydia, 22:163-207.
10. COOKE, W. B. 1961. The cyphellaceous fungi. A study of the Porotheleaceae. Sydowia, Ann. Mycol., IV Beiheft, xv + 144 pp.
11. CORNER, E. J. H. 1932a. A Fomes with two systems of hyphae. Trans. Brit. Mycol. Soc., 17:51-81.
12. CORNER, E. J. H. 1932b. The fruiting body of Polystictus xanthopus Fr. Ann. Bot., 46:71-111.
13. CORNER, E. J. H. 1950. A monograph of Clavaria and allied genera. xv + 740 pp. 298 text figs. 16 col. pls. Oxford University.
14. EFTIMIU, P. and S. KHARBUSH. 1927. Recherches histologiques sur les Exobasidiées. Rev. path. veg. ent. agr., 14:62-88.
15. GADD, C. H. and C. A. LOOS. 1950. Further observations on spore growth of Exobasidium vexans. Trans. Brit. Mycol. Soc., 33:19-21.
16. GILBERTSON, R. L. 1961. Polyporaceae of the Western United States and Canada. I. Trametes Fries. Northwest Science, 35:1-20.

17. GRAAFLAND, W. 1953. Four species of Exobasidium in pure culture. Acta Bot. Neerl., 1:516-522.
18. GRAAFLAND, W. 1960. The parasitism of Exobasidium japonicum Shir. on Azalea. Acta. Bot. Neerl., 9:347-379.
19. GRAY, W. D. 1959. The relation of fungi to human affairs. xiii + 510 pp. 191 figs. Henry Holt and Co., New York.
20. LOWE, J. L. 1942. The Polyporaceae of New York state (except Poria). New York State Coll. For. Syracuse. University Tech. Publ., 60:1-128, Figs. 1-2.
21. LOWE, J. L. 1946. The Polyporaceae of New York state (the genus Poria). New York State Coll. For. Syracuse University Tech. Publ., 65:1-91. 20 figs.
22. LOWE, J. L. and R. L. GILBERTSON. 1961. Synopsis of the Polyporaceae of the southeastern United States. Jr. El. Mitchel Sci. Soc., 77:43-61.
23. MARTIN, G. W. 1961. Key to the families of fungi. In Dictionary of the fungi, pp. 497-517. G. C. Ainsworth. Commonwealth Mycological Institute, Kew.
24. NOBLES, MILDRED K. 1958. Cultural characters as a guide to the taxonomy and phylogeny of Polyporaceae. Can. Jr. Bot., 36:883-926.
25. OVERHOLTS, L. O. 1953. The Polyporaceae of the United States, Alaska, and Canada. (Prep. for publ. by J. L. Lowe.) xiv + 466 pp. 132 pls. Frontisp. University of Michigan Press, Ann Arbor.
26. SAVILE, D. B. O. 1959. Notes on Exobasidium. Can. Jr. Bot., 37:641-656.
27. TEIXEIRA, A. R. 1962. The taxonomy of the Polyporaceae. Biol. Rev., 37:51-81.

F. GLOSSARY:

1. Holobasidium (pl. holobasidia: Gr. holos = entire, i. e. not divided - basidion = a small base): a simple, non-septate and not deeply divided basidium bearing usually four basidiospores on sterigmata.
2. Trama (pl. tramae; L. trama = woof): the fungal tissue composing the pileus or bearing the hymenium of the Homobasidiomycetidae.

Class Basidiomycetes – Sub-Class Homobasidiomycetidae – Series Hymenomycetes

Order: Agaricales

A. GENERAL REMARKS:

1. The Agaricales include the boletes and the mushrooms. The basidia line the tubes or gills (lamellae) of fleshy sporophores.

2. The order is variously sub-divided into from two to fifteen families. We shall study three: Boletaceae, Russulaceae, and Agaricaceae.

B. PROCEDURE:

Family: Boletaceae

Genus: Boletus

3. The Boletaceae are fleshy fungi which resemble mushrooms but which bear their hymenia in pores (tubes). They differ from the Polyporaceae in that, in most species, the hymenia of the Boletaceae are easily separable from the fleshy pileus.

4. Examine any species of the genus Boletus which is available and note the texture of the cap and stem, the color, and the consistency. Observe that the layer of tubes is easily separable from the main portion (context) of the pileus.

Family: Russulaceae

5. The presence of sphaerocysts in the trama of the pileus is the distinguishing character of this family.

Genera: Russula, Lactarius

6. Examine various species of Russula which may be available. Note the fragility of the stalk, the color of the stalk and the pileus, and the arrangement and length of the gills. Examine the spores under the oil immersion objective and note any markings which may be present.

7. If freshly collected specimens of Lactarius are available make a sharp cut across the gills with a sharp scalpel and note the color and consistency of the exudate.

8. Examine a portion of the trama of the pileus of any species of Russula or Lactarius. Note the presence of sphaerocysts.

Family: Agaricaceae

Genera: Amanita, Armillariella, Pluteus, Pholiota, Cortinarius, Agaricus, Coprinus

9. Mount some fresh mycelium which bears clamp connections and study these structures under the oil immersion objective.

10. Observe the rhizomorphs of Armillariella mellea growing on wood. Study prepared slides showing cross sections and longitudinal sections of these structures.

11. Examine large pieces of rotting wood or cultures containing the mycelium of Armillariella mellea. Darken the room completely and note that the wood glows in the dark. This is due to the luminescence of the mycelium of this fungus.

12. Study the sporophore (basidiocarp) of Agaricus campestris, the cultivated mushroom. Observe the stipe, the pileus, the veil, the annulus, and the lamellae. Observe a sporophore of the genus Amanita and note the volva.

13. Study prepared slides of a species of Coprinus showing cross sections of gills. Find the basidia showing sterigmata with basidiospores attached. Distinguish between the trama and the hymenium. Note the structure of the trama in this genus which is characteristic of that of most Agaricaceae. Observe the abundant cystidia in the hymenium.

14. If fresh mushrooms are available choose a well formed sporophore of any species and prepare a spore print as follows: with a sharp razor blade cut off the stem squarely with the edge of the gills. Place mushroom cap down on a piece of white paper. Cover with a bell jar or other cover and allow to stand for a number of hours or until the next laboratory period. Lift the bell jar and carefully remove the mushroom cap. Note the spore print and determine the color of the spores.

15. Study as many genera of mushrooms as time and material will permit and identify a number of species using any one of the standard keys.

C. QUESTIONS:

1. Characterize the Agaricales. Compare and contrast with the Polyporales.
2. How do the Boletaceae differ from the other Agaricales?
3. Discuss the mycorrhizal relationships of the Boletaceae and Russulaceae.
4. Construct a simple key to the families of the Agaricales.
5. What are hallucinogenic mushrooms? In what family do the majority of these fungi belong?
6. What are fairy rings? Explain their formation.
7. How would you distinguish between edible and poisonous mushrooms?

D. TEXT REFERENCES:

Alexopoulos -- pp. 504-520.
Bessey -- pp. 466-529.

E. SELECTED REFERENCES:

1. CHARLES, VERA. 1946. Some common mushrooms and how to know them. Rev. ed. U. S. Dept. Agr. Circ. 143. 60 pp. 49 figs.
2. CHRISTENSEN, C. M. 1943. Common edible mushrooms. x + 124 pp. 62 figs. 4 col. pls. University of Minnesota Press, Minneapolis.

3. CHRISTENSEN, C. M. 1955. Common fleshy fungi. vii + 246 pp. Illustr. Burgess Publishing Co., Minneapolis
4. CHRISTENSEN, C. M. 1961. The molds and man. Ed. 2. viii + 238 pp. 7 figs. 14 pls. University of Minnesota Press, Minneapolis.
5. COKER, W. C. and ALMA H. BEERS. 1943. The Boletaceae of North Carolina. viii + 96 pp. 65 pls. (5 col.) Col. Frontisp. University of North Carolina Press, Chapel Hill.
6. COKER, W. C. and ALMA H. BEERS. 1951. The stipitate hydnums of the Eastern United States. viii + 211 pp. 60 pls. University of North Carolina Press, Chapel Hill.
7. DENYER, W. B. G. 1960. Cultural studies of Flammula alnicola Fr. Kummer and Flammula conissans (Fr.) Gillet. Can. Jr. Bot., 38:909-920.
8. GRAHAM, V. O. 1944. Mushrooms of the Great Lakes region. Chicago Acad. Sci., Spec. Bull. 5. 390 pp. 49 pls.
9. GRAY, W. D. 1959. The relation of fungi to human affairs. xiii + 510 pp. 191 figs. Henry Holt and Co., New York.
10. HEIM, R., and R. G. WASSON. 1957 (1958). Les champignons hallucinogenes du Mexique. 322 pp. 79 figs. (14 in color.) Mus. Hist. Nat., Paris.
11. HERRICK, J. A. 1948. Mushrooms - To eat or not to eat. School Sci. Math., 48:679-685.
12. HESLER, L. R. 1960. Mushrooms of the great Smokies. xii + 289 pp. Illustr. University of Tennessee Press, Knoxville.
13. KAUFFMAN, C. H. 1918. The Agaricaeae of Michigan. Mich. Geol. Biol. Survey Pub. 26, Biol. Serv. 5. Vol. 1. 924 pp. 4 figs. Vol. 2. 10 pp. 172 pls.
14. KRIEGER, L. C. C. 1936. The mushroom handbook. xiv + 512 pp. 126 figs. 32 col. pls. The Macmillan Co. New York.
15. LOQUIN, M. 1956. Petite flore des champignons de France. Vol. 1. 377 pp. 8 figs. 28 pls. Published by the author, Paris.
16. MARTIN, G. W. 1961. Key to the families of fungi. In Dictionary of the fungi, pp. 497-517. G. C. Ainsworth. Commonwealth Mycological Institute, Kew, Surrey.
17. MC ILVAINE, C. 1900. One thousand American fungi. xxxvii + 704 pp. 181 pls. The Bowen-Merrill Co. Indianapolis.
18. PANTIDOU, MARIA E. 1961a. Cultural studies of Boletaceae Can. Jr. Bot., 39:1149-1162.
19. PANTIDOU, MARIA E. 1961b. Carpophores of Phlebotus sulphureus in culture. Can. Jr. Bot., 39:1163-1167.
20. PAPAZIAN, H. P. 1950. A convenient method of growing fruiting bodies of a bracket fungus. Bot. Gaz. 112:138.
21. PAPAZIAN, H. P. 1950. Physiology of the incompatibility factors in Schizophyllum commune. Bot. Gaz. 112:143-163.
22. PILAT, A. Mushrooms. 340 pp. Illustr. 120 col. pls. Spring Books, London.
23. POMERLEAU, R. 1951. Mushrooms of Eastern Canada and the United States. 302 pp. 58 figs. 5 col. pls. Les Editions Chanteclers. Montreal.
24. ROMAGNESE, H. 1956-1960. Nouvelle atlas des champignons. 3 vols. Soc. Mycol., Paris.
25. SINGER, R. 1949. The "Agaricales" (Mushrooms) in modern taxonomy. Lilloa, 22:5-832.
26. SINGER, R. 1958. Mycological investigations on teonánacatl, the Mexican hallucinogenic mushroom. I. Mycologia, 50:239-261.
27. SINGER, R. and A. P. L. DIGILIO. 1960. Los boletaceas de Sudamerica tropical. Lilloa, 30:141-164.
28. SINGER, R. and A. H. SMITH. 1958. Mycological investigations on teonánacatl, the Mexican hallucinogenic mushroom. II. Mycologia, 50:262-303.
29. SMITH, A. H. 1938. Edible and poisonous mushrooms of southeastern Michigan. Cranbrook Inst. Sci. Bull. No. 14. 71 pp. 15 pls. Frontisp.
30. SMITH, A. H. 1949. Mushrooms in their natural habitats. Vol. I, Text. xiv + 626 pp. Vol. II, 231 stereochromes for Viewmaster. Sawyer's Inc. Portland, Oregon.
31. SMITH, A. H. 1958. The mushroom hunter's field guide. 197 pp. Illustr. University of Michigan Press, Ann Arbor.
32. SNIDER, P. J. and J. R. RAPER. 1958. Nuclear migration in the basidiomycete Schizophyllum commune. Am. Jr. Bot., 45:538-546.
33. TAKEMARU, T. 1961. Genetical studies on fungi. X. The mating system in hymenomycetes and its genetical mechanism. Biol. Jr. Okayama Univ., 7:133-211.
34. TAYLOR, N. 1949. Flight from reality. 237 pp. Duell, Sloan, & Pearce, New York.
35. WASSON, R. G. 1959. The hallucinogenic mushrooms of Mexico: an adventure in ethnomycological exploration. Trans. N. Y. Acad. Sci., 21:325-339.
36. WASSON, R. G. 1961. The hallucinogenic fungi of Mexico. Harvard Bot. Mus. Leaflet, 19:137-162.
37. WASSON, VALENTINA P. and R. G. WASSON. 1957. Mushrooms, Russia, and history. 2 vols. xxi + 433 pp. Illust. (Many color plates.) Pantheon Books, New York.
38. WHITEHOUSE, H. L. K. 1949a. Heterothallism and sex in the fungi. Biol. Rev. Cambridge Phil. Soc., 24:411-447.
39. WHITEHOUSE, H. L. K. 1949b. Multiple-allelmorph heterothallism in the fungi. New Phytol., 48:212-244.

F. GLOSSARY:

1. Annulus (pl. annuli; L. annulus = ring): the ring found on the stem of certain species of mushrooms. Remnant of the inner veil.
2. Cystidium (pl. cystidia; Gr. kystis = bladder + -idion = dimin. suffix): a large sterile structure in the hymenium of a basidiomycete.
3. Lamella (pl. lamellae; L. lamina = plate, dimin. form): a plate-like structure (gill) on which some Basidiomycetes produce their basidia.
4. Sphaerocyst (Gr. sphaira = sphere + kystis = bladder): sphaerical cells present in the trama of some Agaricaceae such as *Russula*.
5. Stipe (L. stipes = a post): stem.
6. Veil (L. velum = veil): a thin, veil-like membrane which covers the gills or the entire sporophore of certain members of the Agaricales. Upon expansion of the mushroom the veil tears and remnants appear in the form of a ring, scales, curtain, or volva. The Inner Veil covers the gills.
7. Volva (pl. volvae; L. volva = covering): a cup at the base of the stem of certain mushrooms.

Class Basidiomycetes – Sub-Class Homobasidiomycetidae – Series Gasteromycetes

A. GENERAL REMARKS:

1. The Gasteromycetes produce their basidia in fruiting bodies which often remain completely closed, the spores being liberated when the peridium weathers away or is accidentally broken. Some species, however, expose their spores to the atmosphere, but only after the spores are completely mature.

2. The fungi in this series are classified into a large number of orders, of which the following will be discussed: Lycoperdales (puffballs and earthstars), Sclerodermatales (hard rind puffballs), Phallales (stinkhorns), Nidulariales (bird's nest fungi).

B. PROCEDURE:

Order: Lycoperdales

3. In the Lycoperdales the gleba is powdery at maturity consisting of usually pale, small spores, and of capillitium. In the early stages the basidia form a hymenium, lining minute chambers of the gleba. We shall study representatives of two of the six families: the Lycoperdaceae (puffballs) and the Geastraceae (earthstars).

Family: Lycoperdaceae

Genera: Lycoperdon, Calvatia

4. Examine a sporophore of Lycoperdon and one of Calvatia. Note the ostiole of the inner peridium in the former and the absence of an ostiole in the later. Note the powdery gleba in both.

5. Mount a small portion of the gleba in lacto-phenol and heat. Observe the spores and capillitium.

Family: Geastraceae

Genus: Geastrum

6. Examine specimens of Geastrum umbilicatum or other species and note the splitting of the outer peridium into a star-shaped base on which the spore sac (inner peridium) rests. Note also the mouth of the inner peridium through which the spores escape.

7. Mount a portion of the gleba and study the spores and the capillitium.

Order: Sclerodermatales

8. The Sclerodermatales differ from the Lycoperdales in their larger, usually dark spores, and in that the gleba is not separated into distinct chambers lined with hymenium. Smith (1951) divides the order into 7 families. We shall discuss three of these briefly: the Sclerodermataceae, Tulostomataceae and Astraeaceae.

Family: Sclerodermataceae

Genus: Scleroderma

9. Examine specimens of Scleroderma aurantium, the most common species in this family. Cut a basidiocarp longitudinally in two portions. Note the simple, hard beautifully ornamented peridium, and the compact, dark gleba.

10. Mount a portion of the gleba and study the spores.

Family: Tulostomataceae

Genus: Tulostoma

11. These are the so-called stalked puffballs. Examine specimens of any species of Tulostoma and note the spore case, the stalk, and the mycelial bulb at the base of the stalk.

12. Mount a portion of the gleba. Study the spores and the capillitium.

Family: Astraeaceae

Genus: Astraeus

13. Astraeus resembles Geastrum of the Geastraceae (Lycoperdales) but differs from it in that the basidia of Astraeus are not arranged in a hymenium.

14. Examine a dried sporophore of Astraeus. Place the specimen in the sink and permit water to drip on it from the faucet. Observe the puffing of the spores as the water drops strike the softened inner peridium. Observe and note the changes taking place in the outer peridium.

Order: Phallales

15. The mature spores of the Phallales, enmeshed in a foul-smelling, greenish, gelatinous substance, are exposed at maturity on the surface of a horn-like or basket-like receptacle. There are two families in North America: the Phallaceae and the Clathraceae.

Family: Phallaceae

Genera: Phallus, Mutinus, Dictyophora

16. Examine representatives of each of the above genera and note the differences in structure and color. Observe the volva in each case, and the elaborate indusium in the genus Dictyophora.

Family: Clathraceae

Genus: Clathrus

17. If available, study a mature specimen of Clathrus. Note the basket-like receptacle which carries the gelatinous gleba on the surface of its ribs.

Order: Nidulariales

18. In the Nidulariales the gleba may form a single chamber which separates from the peridium and is violently discharged (family Sphaerobolaceae), or there may be a number of glebal chambers each of which separates as a hard, waxy, lentil-shaped peridiole (family Nidulariaceae).

Family: Nidulariaceae

Genera: Nidula, Nidularia, Crucibulum, Cyathus

19. Examine the basidiocarps of each of the above genera and note the shape and color of the basidiocarp, the shape and color of the peridioles, and the presence or absence of a funiculus. If possible, note how the peridium of each genus dehisces.

20. Soak a basidiocarp of Cyathus or Crucibulum in water for a few minutes and with a sharp pair of forceps grasp one of the peridioles and gently pull so as to unravel the funiculus. Note the length to which you can stretch this cord.

21. Remove a peridiole from the basidiocarp of any species of Cyathus, dip it for a few seconds in a 1:500 solution of $HgCl_3$, wash it thoroughly in sterile distilled water, and plant it on corn meal agar or other medium which supports good fungal growth. Observe from time to time for growth and fruiting.

C. QUESTIONS:

1. How do the Gasteromycetes differ from the Hymenomycetes?
2. What is the peridium? The gleba?
3. Name and describe the four main types of basidiocarp development in this series.
4. What are the "glebal chambers"? In what types of development are they found?
5. Describe the development of the basidiocarp of Lycoperdon. (Consult Ritchie, 1948)
6. How are the spores of the Gasteromycetes disseminated? How does this method differ from that which occurs in the Hymenomycetes?
7. What is the capillitium? The indusium? The receptacle? What are their functions?
8. Of what possible value to the fungus is the bad odor characteristic of the Phallales?
9. What are peridioles? How are they disseminated? (Consult Brodie, 1951)
10. Describe the mechanism of glebal discharge in Sphaerobolus. (Consult Walker, 1927)

D. TEXT REFERENCES:

Alexopoulos -- pp. 520-531.
Bessey -- pp. 530-571.

E. SELECTED REFERENCES:

1. BRODIE, H. J. 1948. Tetrapolarity and unilateral diploidization in the bird's nest fungus Cyathus stercoreus. Am. Jour. Bot. 35:312-320.
2. BRODIE, H. J. 1948. Variation in fruit bodies of Cyathus stercoreus produced in culture. Mycologia, 40:614-626.
3. BRODIE, H. J. 1951. The splash-cup dispersal mechanism in plants. Can. Jour. Bot. 29:224-234.
4. BRODIE, H. J. 1955. Morphology and culture characteristics of a highly aberrant Cyathus. Am. Jr. Bot., 42:168-176.
5. BRODIE, H. J. 1956. The structure and function of the funiculus of the Nidulariaceae. Svensk Bot. Tid., 50:142-162.
6. BRODIE, H. J. and R. W G. DENNIS. 1954. The Nidulariaceae of the West Indies. Trans. Br. Mycol. Soc., 37:151-160.
7. BULMER, G. S. and E. S. BENEKE. 1961 (1962). Studies on Calvatia gigantea. I. Germination of the Basidiospores. Mycologia 53:123-136.
8. COKER, W. C. and J. N. COUCH. 1928. The Gasteromycetes of the Eastern United States and Canada. ix + 201 pp. 123 pls. University of North Carolina Press. Chapel Hill.
9. CONARD, H. S. 1915. The structure and development of Secotium agaricoides. Mycologia, 7:94-104.
10. CUNNINGHAM, D. H. 1924. The structure and development of two New Zealand species of Secotium. Trans. Brit. Mycol. Soc. 10:216-224.
11. CUNNINGHAM, D. H. 1927. The development of Geaster velutinus. Trans. Brit. Mycol. Soc. 12:12-20.
12. DISSING, H. and M. LANGE. 1961. The genus Geastrum in Denmark. Bot. Tid., 57:1-27.
13. HERRICK, J. A. 1950. Hatching stinkhorn eggs in the laboratory. Turtox News, Vol. 28, No. 1. 3 pp. 2 figs.
14. LANDER, CAROLINE A. 1933. The morphology of the fruiting body of Lycoperdon gemmatum. Am. Jour. Bot. 20:204-215.
15. LLOYD, C. G. 1902. The Geasters. Mycol. Writings 1: (1898-1905). 1-44.
16. LONG, W. H. and SULTAN AHMAD. 1947. The genus Tylostoma in India. Farlowia, 3:225-267.
17. LUCAS, E. H., ET AL. 1959. Production of oncostatic principles in vivo and in vitro by species of the genus Calvatia. Antibiot. Annual, 1958-1959. Med. Encycl. Inc., New York.
18. MARTIN, G. W. 1927. Basidia and spores of the Nidulariaceae. Mycologia, 19:239-247.
19. MARTIN, G. W. 1961. Key to the families of fungi. In Dictionary of the fungi, pp. 497-517. G. C. Ainsworth. Commonwealth Mycological Institute, Kew.
20. RITCHIE, DON. 1948. The development of Lycoperdon oblongisporum. Am. Jour. Bot. 35:215-219.
21. ROLAND, J. F., ET AL. 1960. Calvacin: A new antitumor agent. Science, 132:1897.
22. SEDLMAYR, M., E. S. BENEKE, and J. A. STEVENS. 1961 (1962). Studies on Calvatia gigantea. I. Germination of the Basidiospores. Mycologia 53:98-108.
23. SMITH, A. H. 1951. Puffballs and their allies in Michigan. xi + 131 pp. 43 pls. University of Michigan Press. Ann Arbor.
24. WALKER, LEVA B. 1927. Development and mechanism of discharge in Sphaerobolus iowensis n. sp. and S. stellatus Tode. Jour. Elisha Mitchell Sci. Soc. 42:151-178.
25. WALKER, LEVA B. 1940. Development of Gasterella lutophila. Mycologia, 32:31-42.
26. WALKER, LEVA B. and E. N. ANDERSEN. 1925. Relations of glycogen to spore ejection. Mycologia, 17:154-159.
27. WHITE, V. S. 1902. The Nidulariaceae of North America. Bull Torrey Bot. Club, 29:251-280.
28. WRIGHT, J. E. 1949. Los "Gasteromycetes" del museo argentino de sciencias naturales "Bernardino Rivadavia." I. Phallales. Com. Inst. Nac. Inv. Science. Nat., 1, 2, 15 pp.
29. WRIGHT, J. E. 1955. Evaluation of specific characters in the genus Tulostoma Pers. Papers Mich. Acad. Sci., Arts, Lett., 40:79-87.
30. WRIGHT, J. E. 1956. Clave para la identificación de los géneros de Gasteromycetes argentinos. Holmbergia, 5: 45-54.
31. ZELLER, S. M. 1948. Notes on certain Gasteromycetes, including two new orders. Mycologia, 40:639-668.

F. GLOSSARY:

1. Funiculus (pl. funiculi; L. funiculus = a small cord): a thin cord by means of which the peridioles of some Nidulariales are attached to the basidiocarp which bears them.
2. Gleba (pl. glebae; L. gleba = clod): the inner, fertile portion of the fruiting body of the Gasteromycetes.
3. Indusium (pl. indusia; L. indusium = undergarment): a skirt-like structure hanging from the receptacle of the expanded fruiting body of Dictyophora.
4. Peridiole (Gr. peridion = small leather purse + L. -olum = dimin. suffix): the glebal chamber of the Nidulariaceae which has a hard, waxy wall of its own; contains the basidiospores but acts as a propagating unit as a whole.
5. Receptacle (L. receptaculum = container): that portion of the expanded sporophore of the Phallales which bears the gleba.

Form-Class Lichenes

A. GENERAL REMARKS:

1. The Lichenes (lichens) are composed of a large group of dual organisms in which fungi and algae live in intimate association, forming characteristic thalli. In most lichens, the fungal component (mycobiont) is an ascomycete; in a few lichens, it is a basidiomycete.

2. The ascomycetous lichen fungi belong either to the sub-class Loculoascomycetidae, or to the sub-class Euascomycetidae. In these sub-classes we place a number of lichen form-orders and form-families. These are dealt with in more detail in the references on lichens listed below.

Form-order: Lecanorales (Gymnocarpae)

A. GENERAL REMARKS:

3. The Lecanorales produce apothecia on the surface of the thallus. Some of the most common lichen genera belong to this group.

B. PROCEDURE:

Form-family: Cladoniaceae
Form-genus: Cladonia

4. Examine the squamulose type thallus with hollow, brittle, elongate to cup-shaped branches (podetia). This type of thallus is intermediate in structure between the foliose and crustose types. Make a section through the thallus and mount in KOH. Locate the upper cortex, the algal layer, and the medulla.

5. Locate the apothecia at the tips of some of the podetia or branch thalli. Make a longitudinal section through a podetium and mount it in KOH. Locate the epithecium, hymenium, hypothecium, algal layer, medulla, and lower cortex.

6. Using the proper key in Hale's Lichen Handbook, identify several species of Cladonia. Chemical tests are very important in identifying some species.

7. Study and familiarize yourself with several species of Cladonia, such as C. crystatella (brush red coats) with its red apothecia, C. uncialis, which produces usnic acid, and C. rangiferina (reindeer moss).

Family: Physciaceae
Genus: Physcia

8. This is a common form-genus of small, ashy-white, foliose lichens often growing on roadside trees. Apothecia are found frequently in this form-genus. Color tests with KOH on the cortex and medulla are important in separating the species. The cortex should show a yellow color in KOH.

9. Make a cross section of an apothecium of Physcia sp., mount in KOH, and look for the hymenium, algal cells, and the brown, two-celled ascospores.

Genus: Umbilicaria

10. Make a cross section through the thallus of a species of Umbilicaria to see the relationship of the alga and the fungus. Find the cortex, algal layer (green), medulla, lower cortex, and the rhizinae. Check the color reaction of the thallus with small drops of KOH and p-phenylene diamine.

11. Collect some fresh material by removing some of the foliose or fruticose species from trees or stones and bring into the laboratory for identification. Using Hale's Lichen Handbook (pages 103-110), try to identify the genera to which the specimens you have collected belong.

C. QUESTIONS:

1. What classes and orders of fungi are most closely related to lichens?
2. Diagram the typical structure of a lichen thallus.
3. What are economic uses of the lichens?
4. Discuss the relationships between the association of the fungus and the alga in the lichen thallus.

D. TEXT REFERENCES:

Alexopoulos -- pp. 539-543.
Bessey -- pp. 215-223.
Hale -- pp. 1-178.

E. SELECTED REFERENCES:

1. AHMADJIAN, V. 1962. Investigations on lichen synthesis. Am. Jr. Bot., 49:277-283.
2. AHMADJIAN, V. and E. HENRICKSON. 1959. Parasitic relationship between two culturally isolated and unrelated lichen components. Science, 130:1251.
3. FINK, B. 1960. The lichen flora of the United States. Completed for publication by Joyce Hedrick. xii + 426 pp. 4 figs. 47 pls. University of Michigan Press, Ann Arbor. (Reprint of the 1935 edition with a new foreword by by Alexander Smith.)
4. HALE, M. E. 1958. Vitamin requirements of three lichen fungi. Bull. Torrey Bot. Club, 85:182-187.
5. HALE, M. E. 1961. Lichen handbook. x + 178 pp. 58 figs. 20 pls. Publ. No. 4434. Smithsonian Institution, Washington.
6. LLANO, G. A. 1944. Lichens, their biological and economic significance. Bot. Rev., 10:1-65.
7. LLANO, G. A. 1951. Economic uses of lichens. Ann. Rpt., Smithsonian Institution. 1950, pp. 385-422.
8. SMITH, D. C. 1960. Studies in the physiology of lichens. 2. Absorption and utilization of some simple organic nitrogen compounds by Peltigera polydactyla. Ann. Bot., n. s., 24:172-185.
9. STARK, J. B. and E. WALTER, 1950. Method of isolation of usnic acid from Ramalina reticulata. Jr. Am. Chem. Soc., 72:1819-1820.
10. THOMSON, J. W. 1958. An illustrated key to common lichens. Audubon Soc. Nat. Bull., ser. 27, no. 2.

F. GLOSSARY:

1. Algal layer: The layer of algal cells in the lichen thallus.
2. Crustose (L. crusta = crust): a flat, crust-like lichen thallus whose lower surface adheres firmly to the substratum.
3. Epithecium (pl. epithecia; Gr. epi = on + theke = container): a layer of tissue above the asci in an apothecium, formed by the fusion of the tips of the paraphyses.
4. Foliose (L. folium = leaf): a leaf-like lichen thallus.

5. Medulla (pl. medullae; L. medulla = bone marrow): the inner hyphal layer in a lichen thallus.
6. Mycobiont (Gr. mykes = mushroom, fungus + bios = life): the fungal component of a lichen.
7. Phycobiont (Gr. phykos = seaweed, alga + bios = life): the algal component of a lichen.
8. Podetium (pl. podetia; Gr. pous = foot): a stalk-like structure on a lichen thallus, with an apothecium at its tip.
9. Rhizinae (sing. rhizina; Gr. rhiza = root): the rhizoids of a lichen thallus.
10. Squamulose (L. squama = scale): covered with minute scales.

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